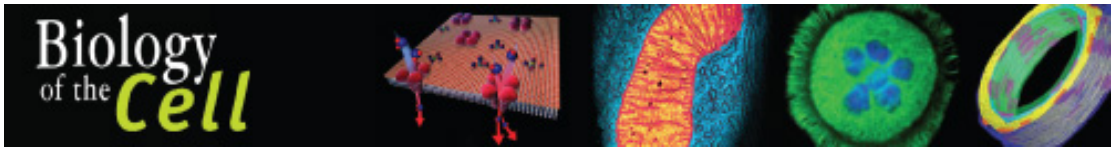

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MAPping out distribution routes for kinesin couriers

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ABSTRACT

In the crowded environment of eukaryotic cells, diffusion is an inefficient distribution mechanism for cellular components. Long-distance active transport is required and is performed by molecular motors including kinesins. Furthermore, in highly polarized, compartmentalized and plastic cells such as neurons, regulatory mechanisms are required to ensure appropriate spatio-temporal delivery of neuronal components. The kinesin machinery has diversified into a large number of kinesin motor proteins (Kifs) as well as adaptor proteins that are associated with subsets of cargo. However, many mechanisms contribute to the correct delivery of these cargos to their target domains. One mechanism is through motor recognition of subdomain-specific microtubule (MT) tracks, sign-posted by different tubulin isoforms, tubulin post-translational modifications (PTMs), tubulin GTPase activity and MT associated proteins (MAPs). With neurons as a model system, a critical review of these regulatory mechanisms is presented here, with particular focus on the emerging contribution of compartmentalised MAPs. Overall, we conclude that - especially for axonal cargo - alterations to the MT track can influence transport, although *in vivo*, it is likely that multiple track-based effects act synergistically to ensure accurate cargo distribution.

The size, functional compartmentalisation and polarity of eukaryotic cells present major challenges for sorting and distribution of subcellular components. Transport by the microtubule (MT)-based motors dynein and members of the kinesin superfamily play a significant role in long-range transport and specific distribution of cellular parcels of various sizes, from mRNA particles to mitochondria. Various mechanisms operate to ensure delivery to the correct location, including: 1) cargo-dependent localisation via adaptor proteins; 2) motors with different speeds and directionalities that take particular routes through the cell; 3) MT track-based road blocks and diversions. Discussions of motor-cargo specification have been presented elsewhere (e.g. Akhmanova and Hammer, 2010). A number of recent studies are beginning to provide mechanistic insight into the way in which the kinesin-MT interaction can be modulated - including through the action of MT Associated Proteins (MAPs) - to achieve compartmentalized and polarized cargo distribution, and this review will focus on this subject.

Introduction: Kinesin family members target specific cargo to particular cellular subdomains

In contrast to the paucity of cytoplasmic dynein heavy chains (two genes identified in mice and humans; Pfister et al., 2006), the kinesin repertoire in mammals boasts 45 kinesin heavy chain (KHC) genes, with additional protein variants derived from splice variants (Miki et al., 2003). Kinesin family members are somewhat confusingly referred to as both 'Kifs' and 'kinesins' with different numbering systems. For functional clarity and precision we will use the Kif nomenclature in this review. The well-conserved Kif motor domains define the Kif superfamily, have MT-stimulated ATPase activity and typically operate in the context of homo- or hetero-dimers (Hirokawa et al., 2010; Verhey and Hammond, 2009). The Kif tail domains are divergent and can bind different cargos directly, whilst Kif association with cargo-binding adaptors, including kinesin light chains (KLCs), enables further specific freight interactions.

Kifs use polar, tubular polymers of α/β tubulin heterodimers – MTs – as tracks. MTs are intrinsically dynamic, and although the so-called minus-ends are typically stabilised at the cell's MT organising centre, the opposite plus-ends exhibit phases of polymerization or depolymerisation within the cytoplasm that are precisely regulated *in vivo* (Janke and

Bulinski, 2011; Wade, 2009). Regulation of MT dynamics must necessarily be coordinated with requirements for MT-based transport. In general, whilst transport via dynein is towards the minus-end of MTs (retrograde transport), the Kifs of the subtype N-Kif (with an N-terminal motor domain) are solely responsible for MT-based transport towards the plus-end (anterograde transport; Figure 1). A small minority of Kif family members – the C-Kifs (with C-terminal motor domains) – are minus-end directed and involved in crosslinking and sliding MTs during mitosis, (Peterman and Scholey, 2009; Sharp et al., 2000) and differentiated cells (Bananis et al., 2004; Hanlon et al., 1997; Noda et al., 2001; Saito et al., 1997; Xu et al., 2002; Yang et al., 2001). The remaining Kifs, the M-Kifs, have a central motor domain, act as MT depolymerases and control MT organisation *in vivo* both during cell division and in interphase (Hirokawa et al., 2009; Wordeman, 2005).

The functions of individual Kifs and the cargo they carry have been studied in a wide variety of cell contexts. In the mitotic spindle, for example, the multiple Kifs that operate as part of this complex cellular machinery manoeuvre their cargo in a highly orchestrated manner to ensure accurate segregation of the duplicated chromosomes to each daughter cell (Douglas and Mishima, 2010; Peterman and Scholey, 2009; Sharp et al., 2000). In differentiated cells, the roles of Kifs in carrying different cargos are also emerging (Hirokawa et al., 2009). The existence of different populations of Kif-driven vehicles each with distinct passenger sets could provide a mechanism for restricted and precise distribution of particular cargos in polarized and compartmentalized cells, but this mechanism depends on individual Kifs being dispatched along particular routes to specific destinations.

Below, we will focus primarily on the classic and extensively studied case of neuronal transport. Here, the need for efficient regulatory mechanisms guiding compartment-specific cargo delivery is extreme in many respects. A neuron's large size and high degree of polarization, plasticity, compartmentalization and compartmentalized activity requires direction-restricted transport, often occurring over vast distances and in a manner responsive to discrete spatio-temporal signals. In certain human motor neurons for example, cargos originating in the soma have final destinations more than a metre away in the periphery. Pre- and post-synaptic compartments are defined and dynamically modified by exclusive and on-demand delivery of their specific components, enabling proper neuronal function and plasticity.

The MT array in neurons is very precisely organised, with axonal MTs having their plus-ends located distally, whilst the polarity of MTs in the dendritic compartment is mixed (Baas et al., 1988). Thus, the minus-end (dynein and C-Kifs) and plus-end directed (N-Kifs) motors could by themselves form the basis of a simple system of polarized cargo transport. However, it is now clear that some plus-end directed Kifs can move preferentially into either axons or dendrites to deliver compartment-specific cargo (Byrd et al., 2001; Huang and Banker, 2011; Jacobson et al., 2006; Nakata and Hirokawa, 2003; Nishimura et al., 2004; Saito et al., 1997; Setou et al., 2000; Shi et al., 2004). The compartment-specific movement of individual Kifs can depend on the experimental context (Table 1) and in particular, whether the motor is constitutively active (i.e. tail-less and typically tagged using GFP) or full-length (i.e. a subset could be autoinhibited and not bound to cargo). Nevertheless, the active guidance of a defined subset of transport Kifs (Silverman et al., 2010) preferentially into axons or dendrites (Hirokawa et al., 2010; Huang and Banker, 2011) represents an intriguing example of transport regulation in this cell type, a phenomenon which appears to both establish and maintain polarized neuronal structure and function (Jacobson et al., 2006; Nakata and Hirokawa, 2007). Furthermore, there appear to be mechanisms for halting/releasing some cargos at specific locations in particular conditions, such as Ca^{2+}-dependent pausing of Kif5-mitochondrial complexes at synapses, or enrichment of Kif5, Kif3A and Kif13B motors in the growth cone of developing axons (Horiguchi et al., 2006; Nadar et al., 2008; Nishimura et al., 2004; Yoshimura et al., 2010). For some kinesins, including Kif5s, the motor domain alone is sufficient to define their physiologically relevant and typically axonal localisation, implying that a track-based guidance system for cargo carried by these motors is in operation. However, some usually dendritic cargos actively define their final destination – the "cargo steering" hypothesis – independently of the motors to which they are attached, suggesting that localisation mechanisms not defined by the MT tracks are also in operation (Huang and Banker, 2011; Jenkins et al., 2012; Nakata and Hirokawa, 2003; Setou et al., 2000; Setou et al., 2002).

Are tubulin PTMs road markers for polarized Kif transport?

The molecular basis for the selective distribution of Kif motors and their cargo is critical in understanding the establishment and maintenance of cell polarity, but we are currently very far from understanding this complex phenomenon. The vast majority of

research on this subject has focused on Kif5 (conventional kinesin/kinesin-1), the founding member of the kinesin superfamily. When Kif5 is truncated at its C-terminus to form a constitutively motile dimer, it preferentially localises to the axonal compartment, implying that the interaction between the Kif5 motor domains and the MT tracks are sufficient to define its polar distribution (Nakata and Hirokawa, 2003; Thorn et al., 2000). One possible mechanism is through compartment-specific post-translational modifications of αβ-tubulin – typically on MT-incorporated tubulin rather than on the soluble tubulin pool (Figure 1). Most but not all of these modifications are made to the flexible C-terminal tails (CTTs) of α- or β-tubulin on the MT surface (Figure 1), which themselves are known in general terms to contribute to kinesin motility (Thorn et al., 2000). The effects of a number of post-translational modifications (PTMs) of tubulin on Kif5 MT binding, motility, and selectivity have been investigated (for a longer recent review, see also Garnham and Roll-Mecak, 2012).

α-tubulin acetylation is a prevalent PTM on stable MTs, particularly in neuronal axons, and has been correlated with enhanced Kif5 binding and motility (Konishi and Setou, 2009; Reed et al., 2006). Hyper-acetylation of MTs in all neuronal subcellular compartments via treatment with HDAC inhibitors depolarizes the distribution of Kif5 (Tapia et al., 2010) and its JIP-1 cargo (Reed et al., 2006), at first sight making this modification a compelling candidate for control of polar trafficking. However, the molecular basis for such control is not immediately obvious, since a principal site of α-tubulin acetylation is Lys40, which is located in the lumen of the MT and remote from the kinesin binding site (Nogales et al., 1998; Soppina et al., 2012). In fact, more recent studies suggest that pharmacological perturbation of tubulin acetylation does not affect Kif5 distribution (Hammond et al., 2010; Nakata et al., 2011). Some of these discrepancies may be explained by variations in experimental conditions, including the treatment parameters and age of cultured neurons used, but it should also be noted that HDAC inhibition in the cellular environment can have a number of off target side-effects both on and off the MT, including on MAPs themselves (Cook et al., 2012). Indeed, *in vitro* experiments found no effect of Lys40 acetylation on Kif5 binding, run length or velocity, suggesting that this *alone* cannot directly account for selective axonal Kif5 distribution (Soppina et al., 2012; Walter et al., 2012). Although Kif5 and associated cargo has been shown to move preferentially on acetylated MTs in COS cells

(Cai et al., 2009), cellular Kif5 and its cargo also move on non-acetylated MTs or those MTs marked by detyrosination. Finally, HDAC6 knockout mice develop normally despite widespread tubulin hyperacetylation (Zhang et al., 2008). In summary, tubulin acetylation alone cannot account for axon specific transport by Kif5.

Similarly, detyrosinated α-tubulin is also found mainly in the neuronal axon and polarised movement of Kif5 and/or its cargos along stable cellular MTs can be selectively disrupted both by antibodies to detyrosinated (but not tyrosinated) tubulin (Gurland and Gundersen, 1995) and by siRNA-mediated knockdown of tubulin tyrosine ligase (Ttl) (Konishi and Setou, 2009). Kif5 binds more tightly to detyrosinated MTs *in vitro* (Dunn et al., 2008; Konishi and Setou, 2009; Kreitzer et al., 1999; Liao and Gundersen, 1998) and recognition of this PTM and related polarized transport depends on Kif5's divergent β5-L8 region which is part of the kinesin-MT-binding interface (Figure 2). However, although mice deficient in Ttl exhibit abnormal axonal development (Erck et al., 2005; Marcos et al., 2009), ultimately a polarised morphology develops - which likely requires intact polarized Kif5 transport (Hammond et al., 2010; Jacobson et al., 2006; Konishi and Setou, 2009). Indeed, polarized distribution of Kif5 in neuronal populations cannot be fully accounted for by distribution patterns of detyrosinated tubulin and occurs at a developmental stage preceding any polarized distribution of detyrosination (Hammond et al., 2010; Nakata et al., 2011). Furthermore, in COS cells (Cai et al., 2009) much of Kif5's movement occurs on tyrosinated tubulin MTs.

Total but not partial loss of polyglutamylation of at least β-tubulin inhibits Kif5 MT binding, but a similar inhibition also occurs when *increasing* glutamyl chain length past three units (Larcher et al., 1996; Reed et al., 2006). A negative correlation has been reported between tubulin polyglutamylation and the motility of Kif5 cargo adaptor gephyrin when neuronal activity is enhanced in cultures via glycine receptor blockade (Maas et al., 2009). However, as this effect was found to be cargo-specific, with motility of Kif5 cargo adaptor GRIP-1 unaffected by the same treatment, modulation of the interaction between the MT track and the Kif5 motor domain cannot explain this phenomenon. Interestingly, severe loss of the majority of α-tubulin-polyglutamylation with additional partial loss of β-tubulin-polyglutamylation *in vivo* and *ex vivo* has no obvious effect on Kif5 distribution; instead it specifically prevents the normal motility of the Kif1A motor into all neurites (Ikegami et al.,

2007). Knockdown of the β-tubulin glutamylation enzyme TTLL7 inhibits outgrowth of all neurites in PC12 cells (Ikegami et al., 2006), and it is possible this could be linked to disruption of kinesin motility. Most brain tubulin is polyglutamylated (Audebert et al., 1994) and this type of PTM is not obviously localized to particular parts of the neuron *per se*, although as the available GT335 glutamylated tubulin antibody does not differentiate between α- and β-tubulin modification, or chain lengths longer than a single unit, it is possible these factors vary between different sub-domains (Bonnet et al., 2001; Hammond et al., 2010; Ikegami et al., 2006). However, in non-proliferating non-neuronal cell lines polyglutamylation levels are very low (Bobinnec et al., 1998), but Kif5 still travels along select MTs (Cai et al., 2009). All considered there is as yet no clear evidence that poly-glutamylation acts as a directional cue for Kifs, but it could be important in providing a suitable general track for some motors such as Kif1A (Ikegami et al., 2007; Ikegami et al., 2006), modulating transport dynamics with age (Audebert et al., 1994), or neuronal activity-dependent regulation of some kinesins (Maas et al., 2009).

Tubulin PTMs can accumulate cooperatively, such that manipulation of one PTM can produce off-target effects in others, confounding a straightforward interpretation of measured phenotypes. For example in the report from Ikegami and colleagues (2007) genetically targeted loss of α-tubulin polyglutamylation in mice also produces alterations in tyrosination and β-tubulin polyglutamylation. Although they have received little attention to date, other tubulin PTMs such as polyglycation, phosphorylation, palmitoylation (Fukushima et al., 2009) and ADP-ribosylation (Kaslow et al., 1981) could also influence kinesin motility. While most of the research on MT PTM influence on Kif distribution and localization has focused on Kif5, the distribution of other kinesin superfamily members may be more or less influenced by different MT PTMs. For example, although Ikegami and colleagues (2007) showed that the affinity of Kif5, Kif1A and Kif3A for MTs from mice deficient in α -tubulin polyglutamylation was reduced, only Kif1A was abnormally localized as a result. In COS cells, only Kif5 but not Kif17 or Kif1A move preferentially on acetylated and/or detyrosinated MTs (Cai et al., 2009). An additional source of MT track variation is the range of α- and β- tubulin isotypes (6 or more genes for each in vertebrates) expressed in different cell types, with each isotype varying chiefly in the C-terminal region (Fukushima et al., 2009) and therefore being more or less subject to different combinations of PTMs (Tischfield and Engle, 2010). It

should also be noted that experiments *in vitro* using brain tubulin will typically contain a heterogeneous mixture of tubulin isoforms and PTMs, which is likely to complicate mechanistic interpretations.

Taken together, evidence for a role for tubulin PTMs alone in controlling polarised trafficking is far from conclusive and, at a minimum, it seems very unlikely that a single MT modification can account for all regulated distribution of Kifs. It is more likely that tubulin PTMs act in concert together and with other co-factors (Hammond et al., 2010; Walter et al., 2012). As discussed, diverse mechanisms appear to control differing MT selectivity and cellular distribution of the different Kif family members. Particularly in neurons, structural MAPs are especially abundant and compartmentalised and tubulin PTMs may alter Kif selectivity by altering MT-MAP interactions. Indeed, there are numerous examples of the distribution, or MT interactions, of structural MAPs including MAP1, MAP2 and tau being altered by tubulin PTMs (Bonnet et al., 2001; Boucher et al., 1994; Ikegami et al., 2007; Konishi and Setou, 2009; Larcher et al., 1996; Saragoni et al., 2000; Spiliotis et al., 2008). Conversely, the binding of MAPs to MTs could act upstream to directly or indirectly influence their PTM state; for example, tau binding has been found to positively regulate tubulin acetylation (Perez et al., 2009; Takemura et al., 1992). Compartmentalized MAPs could act indirectly to alter accumulation of MT PTMs and both could thus contribute to selective kinesin distribution. Many MAPs can function to stabilize MTs (Amos and Schlieper, 2005) and tubulin PTMs appear to be consequences rather than modulators of increased MT stability (Khawaja et al., 1988; Palazzo et al., 2003; Paturle-Lafanechere et al., 1994). MAPs could thus play a critical role to promote local 'tagging' of MTs with specific PTMs. However, MAPs also contribute directly to the spatio-temporal localization of specific Kif motors during mitosis (Bieling et al., 2010; Ye et al., 2011) and the regulation of Kif distribution by MAPs in neuronal cells independently of tubulin PTMs will be discussed below.

Regulation of Kifs by the tubulin GTPase cycle

The dynamic properties of MTs arise from the intrinsic GTPase of αβ-tubulin; in solutions of pure tubulin, MTs undergo stochastic switching between phases of growth driven by addition of GTP-bound tubulin and shrinkage driven by GTP hydrolysis within

the lattice (Desai and Mitchison, 1997). *In vivo*, these dynamics are tightly regulated according to cell activity and have recently been shown to be structurally coupled to the activities of plus-end tracking proteins (+TIPs; Akhmanova and Steinmetz, 2008; Montenegro Gouveia et al., 2010). +TIPs cluster at the plus-ends of growing MTs and control local dynamics and subcellular targeting of MT ends. End Binding proteins (EBs) are the master regulators of these dynamic protein hubs and localise to growing MT ends by recognising a conformation of tubulin that depends on GTP hydrolysis within the MT lattice (Maurer et al., 2011; Maurer et al., 2012).

EBs are involved in setting up MT polarity in neurons, and have also been used as experimental tools to visualise neuronal MT plus ends and, thereby, polarity (e.g. Mattie et al., 2010; Stone et al., 2008). However, it has been broadly assumed that 1) MT tracks are specifically stabilised to support long-range cargo and 2) they are composed of GDP-tubulin because GTP hydrolysis in the lattice has already occurred. The assumption of homogeneity with respect to tubulin-bound nucleotide was challenged during the characterisation of an antibody (hMB11) raised against tubulin bound to the non-hydrolysable GTP analogue GTPγS (Dimitrov et al., 2008). Using this antibody, small islands along cellular MTs were labelled, suggesting that microtubule GTPase is biochemically heterogeneous in cells, or at least the conformational consequences of tubulin GTPase are (Kueh and Mitchison, 2009). Dimitrov et al initially proposed that such "GTP islands" would promote MT rescue (recovery from MT shrinkage to growth) at functionally important sites in the cell. However, an additional role for these GTP islands has recently been suggested to contribute to Kif5's polarised axonal localisation (Nakata et al., 2011). hMB11 showed preferential immunolocalisation to the axon and colocalised with Kif5 but not Kif1A motors. In addition, fluorescently tagged Kif5 showed preferential binding to GMPCPP-MTs (a very slowly hydrolysable GTP analogue) as opposed to GDP-MTs *in vitro*, supporting a nucleotide-based mechanism for the *in vivo* binding pattern. Recognition of the underlying MT nucleotide state is also indicated by previous studies that showed that Kif5-driven GMPCPP-MTs move roughly 34% faster than paclitaxel-stabilized GDP-MTs using in vitro multi-motor gliding assays, without alteration of Kif's ATPase rate (Vale et al., 1994), although not all reports support this finding (Moores et al., 2006). The basis of such recognition of – and preferential binding to – "GTP-like" MTs is specified by Kif5's loop 11 region at its MT binding interface

(Figure 2) (Nakata et al., 2011). A sub-nanometer resolution cryo-EM reconstruction of GMPCPP-MTs (Yajima et al., 2012) suggests that Kif5 loop11 is well positioned to sense nucleotide-dependent conformational changes within the MT (Figure 2). Interestingly, these GTPase-related conformational changes in tubulin are similar to those recognised by EBs, even though the primary MT binding sites for these unrelated families of proteins are very different (Maurer et al., 2012).

The tubulin islands recognised by hMB11 are rather sparsely distributed along axons, suggesting that polarisation of neuronal transport is unlikely to be solely determined by the guanine nucleotide state of MTs. In addition, recent experimental evidence from *in vitro* single molecule imaging shows that Kif5 has *shorter* run lengths and unaltered velocity on GMPCPP-MTs compared to paclitaxel-stabilized GDP-MTs (McVicker et al., 2011). In non-neuronal cells Kif5 travels preferentially on sub-populations of MTs, consistent with the existence of distinctive cues on particular MTs (Cai et al., 2009; Dunn et al., 2008), whereas there are no obvious *inter*-MT differences in GTP-tubulin content, but rather only *intra*-MT discontinuities in GTP-tubulin content along the MT length (Dimitrov et al., 2008). However, structural signals arising within the MT lattice due to GTP islands may be amplified by other factors, including tubulin PTMs (in particular Lys-40 α-tubulin acetylation; Yajima et al., 2012) and selective recruitment of MAPs.

The Tau/MAP2 family; obstacles to Kifs?

Tau and MAP2 are homologous MT binding proteins that are abundant in neurons. These MAPs present themselves as particular targets for study in the context of polarised neuronal transport because tau isoforms are predominantly associated with axonal MTs in mature neurons, while MAP2 is restricted to the dendritic compartment (Binder et al., 1985; Deshpande et al., 2008). Tau and MAP2 are very similar in their MT binding domain but differ in their extended “projection domains” which protrude from the MT surface. Tau and MAP2 are highly regulated by phosphorylation at a large number of phosphorylation sites (at least 63 identified; Hanger et al., 2009) inside and outside of the MT binding repeat region, which, amongst other things, can modulate MT association (Figure 3).

Numerous studies have demonstrated that over-expression of tau causes inhibition of Kif5 motility and alters the subcellular distribution of its cargo (Dubey et al., 2008; Ebneth

et al., 1998; Mandelkow et al., 2003; Stamer et al., 2002; Stoothoff et al., 2009; Trinczek et al., 1999). Thus, in primary neuronal cultures or neuronal cell lines, when tau is over-expressed and becomes abnormally distributed, Kif5s and their cargo accumulate in the soma and fail to enter any processes. When added in non-physiological excess to MTs *in vitro*, tau - and to a greater extent MAP2 - inhibit the motility of Kif5 motors and associated cargo along MTs (Hagiwara et al., 1994; Heins et al., 1991; Lopez and Sheetz, 1993; Seitz et al., 2002). At sub-saturating concentrations of MAPs, the effects on Kif5 transport is harder to pin down: some constructs exhibit inhibitory effects on motor attachment and run length (Dixit et al., 2008; McVicker et al., 2011; Seitz et al., 2002), whereas others do not inhibit motility at all (Hagiwara et al., 1994; Seitz et al., 2002; von Massow et al., 1989). In fact, enhancements of single molecule Kif5 run length and attachment frequency by some tau constructs has also been reported (Lopez and Sheetz, 1993; Seitz et al., 2002). Whilst there is a general consensus that velocity is not grossly affected once motors are in motion, contrasting evidence has been collected on whether motility inhibition occurs because of shortening of motor run length by tau or reducing motor attachment events (Seitz et al., 2002; Trinczek et al., 1999). The inhibitory effects of tau on long-distance transport may be particularly acute in cargo carried by multiple motors, the most likely physiologically relevant scenario. For example, Vershinin et al demonstrated that, whereas multiple motors can increase the processivity of a cargo along undecorated MTs by an order of magnitude compared to a single Kif5 motor, tau (especially 3R tau) significantly inhibited this processivity enhancement (Vershinin et al., 2007). Even at low tau/tubulin ratios, tau can reduce multi-kinesin-dependent cargo run length by inhibiting the motor reattachment that is required for processivity enhancement. When only single motors were engaged in motor transport, the inhibitory effect of tau on run length was much reduced. Unsurprisingly, therefore, there is no consensus concerning the molecular mechanism(s) for effects of MAP2/tau on kinesin transport, and they have been variably attributed to: 1) direct competition between motor and tau/MAP binding to the MT (Ebneth et al., 1998; Trinczek et al., 1999); 2) resistance to motor movement by the negatively charged projection domains protruding from the MT (Hagiwara et al., 1994; Lopez and Sheetz, 1993); 3) blockage of motility that is independent of high affinity MT binding (Seitz et al., 2002; Vershinin et al., 2007), possibly due to protein aggregation, either non-specific or by filament formation (Ackmann et al., 2000; Duan and Goodson, 2012; Santarella et al., 2004);

4) disruption of the Kif-cargo interaction by tau (Kanaan et al., 2011; LaPointe et al., 2009; Morfini et al., 2007).

Two different structural models propose that tau/MAP2 binds either to the lumenal surface of MTs (Kar et al., 2003) or the tubulin dimer ridges on the outside of MTs (Al-Bassam et al., 2002). However, the apparently contradictory results can be explained by the experimental conditions of MT assembly used in each study: where paclitaxel is used to stabilize MTs prior to MAP addition, tau binds to the external tubulin ridges of MTs at a site overlapping that of kinesin (Al-Bassam et al., 2002), whereas when MTs are co-assembled with tau without paclitaxel, but rather with the presence of non-hydrolysable GTP analogue GMPCPP, tau binds to the inside lumen of MTs on β-tubulin at a binding site at least partly overlapping that of paclitaxel (Kar et al., 2003). The majority of *in vitro* Kif motility interference studies using tau have utilized pre-formed MTs stabilized with paclitaxel prior to addition of Kif, supporting the interpretation of direct binding competition between Kif and MAP2/tau on the MT surface in these assays, also reinforced by more recent work (McVicker et al., 2011; Peck et al., 2011). One simple conclusion from these findings is that data from experiments relying on MT stabilisation with paclitaxel are artifactual due to paclitaxel blockage of tau's native binding site. However, since GMPCPP-stabilised MTs, are mimics of a GTP-like state, they are also not representative of the majority of cellular MTs which are composed of GDP-tubulin. Instead, it now seems more likely that tau binding to tubulin/MTs is modulated by the nucleotide state of tubulin, which also has an impact on Kif motility. Thus, tau has no effect on Kif transport when paclitaxel and GMPCPP are used together, while it is inhibitory when MTs are stabilized in paclitaxel free, glycerol- and DMSO-containing conditions (McVicker et al., 2011; Peck et al., 2011). That the nucleotide state of tubulin, rather than the presence or absence of bound paclitaxel, modulates the nature of tau binding and Kif motility is consistent with further cryo-EM (Santarella et al., 2004) and binding kinetics (Makrides et al., 2004) studies. Although not yet demonstrated, it is likely that MT binding by other tau family members including MAP2 is also influenced by the nucleotide bound state of the MT, due to high homology in the MT binding domains. In neurons, the MT guanine nucleotide state appears to be somewhat polarized, with higher levels of GTP tubulin in the axon (Nakata et al., 2011); it is possible - though no proven

that this contributes to axonal localisation of tau and that these effects together synergistically contribute to compartmentalized distribution of Kifs.

Whether tau has a major modulatory role in guiding kinesin distribution *in vivo* has been called into question by the observation that tau (Harada et al., 1994) or MAP2 (Teng et al., 2001) knockout mice have no major phenotype. Pathological aggregation and hyperphosphorylation of tau especially in older overexpressing/mutant mice complicates physiological interpretations of tau modulation of motor behaviour (Adams et al., 2009; Duff and Suleman, 2004; Polydoro et al., 2009; Terwel et al., 2005). However, no observable disruption of Kif-based transport in optic axons was reported in tau knockout mice, or in 3-18 month old mice over-expressing human tau (Yuan et al., 2008), or in the *C. elegans* knockout of tau homologue PTL-1 (Tien et al., 2011). It is possible that MAP1b compensates for the absence of tau or MAP2 in these cases because double knockout MAP2/MAP1b or tau/MAP1b mice exhibit abnormal neuronal development, morphology and function (Takei et al., 2000; Teng et al., 2001). However, association of these gross phenotypes to specific alterations of Kif behaviour is premature, especially considering MAP1b and tau share low structural similarity (Amos and Schlieper, 2005) and produce different effects on anterograde transport when depleted from cultured neurons (Jimenez-Mateos et al., 2006).

In studies of the non-neuronal Ltk cell line, over-expression of full-length MAP4 (a non-neuronal MAP related to MAP2/tau) or a truncated construct containing only the MT binding repeats was found to reduce the motility of vesicle transport (Bulinski et al., 1997), and slow the general speed of the cell cycle (Nguyen et al., 1997), consistent with MAP4 interference with kinesin motility. Later *in vitro* gliding assays found that high concentrations of the 5, but not 4 or 3 MT binding repeat isoform of MAP4 inhibited MT gliding on Kif5, with increases in MT pausing and decreased MT run lengths. This effect was seen without MAP4 competing with Kif5 for MT binding by co-sedimentation assay. However, particularly high non-physiological saturating concentrations of MAP4 were required in this study for any clear effect on MT motility. Unfortunately, much like the literature on tau and MAP2, it is hard to draw conclusions about any normal *in vivo* role of MAP4 in regulation of Kifs from experiments using non-physiologically high MAP4 concentrations *in vitro* and *ex vivo*. However, considering some of the more recent work on tau's sensitivity to tubulin-bound nucleotide (McVicker et al., 2011; Peck et al., 2011) and its

tendency to polymerise when bound to MTs (Duan and Goodson, 2012), more physiologically orientated experiments on MAP4 are warranted.

In summary, a lack of clear conclusion from the above meta-analysis of *in vitro* and *ex vivo* work combined with the *in vivo* evidence from transgenic mice suggests that it is unlikely that MAP2, tau and their relatives have direct, prominent and/or specific physiological roles in controlling polarized transport of kinesin motors. For example, whilst polarized transport of Kif5 is one of the initial events in axonal specification, neuronal polarization of tau and MAP2 localizations occur later in development (Boyne et al., 1995; Deshpande et al., 2008; Jacobson et al., 2006; Mandell and Banker, 1996; Nakata and Hirokawa, 2003), and neither are essential for Kif5 polarization (Hammond et al., 2010; Nakata et al., 2011) or axonal specification (DiTella et al., 1996; Harada et al., 1994; Takei et al., 2000). Furthermore, Kif5 itself appears to be involved in the polarized transport of tau (Utton et al., 2005). It is worth noting however that tau family MAPs may act alongside other MAPs in combinatorial fashion and in response to signals, such that modulation of Kifs may only become prominent when a number of MAPs collaborate. For example, MARK kinase activation, which may detach many MAPs from MTs simultaneously (Drewes et al., 1998; Schaar et al., 2004) has enhancing effects on Kif5 based cargo transport (Mandelkow et al., 2003; Mandelkow et al., 2004).

DCX/DCLK1 are required for proper distribution of Kif1A and associated cargo

Doublecortin (DCX) and its close homologue doublecortin-like kinase-1 (DCLK1) contain tandem MT binding 'DC' domains, named N-DC (N-terminal) and C-DC (C-terminal) (Sapir et al., 2000; Taylor et al., 2000), as well as a serine/proline-rich C-terminal domain and, in the case of DCLK1, a kinase domain (Figure 3). The DC domains have a β-grasp-like fold (Cierpicki et al., 2006; Kim et al., 2003) and thus the MT binding domains of DCX and its homologues show no similarity to those of the tau/MAP2 family. 11 members of the DCX family - defined by the presence of at least one DC domain – have now been identified in humans, along with various splicing isoforms (Dijkmans et al., 2010; Reiner et al., 2006). Mutations in the *DCX* gene, many of which cluster within the DC domains (Quelin et al., 2012; Sapir et al., 2000; Taylor et al., 2000), lead to disorders of abnormal brain development such as lissencephaly, with disrupted cortical layering associated with

perturbed neuronal migration (des Portes et al., 1998a; des Portes et al., 1998b; Gleeson et al., 1998; Sossey-Alaoui et al., 1998). In mice, DCX knockout causes only subtle brain abnormalities, whereas the additional knockout of DCLK1 produces severe disruption of cortical layering phenotypes resembling the human disease. This is likely due to functional redundancy between the proteins in mice (Corbo et al., 2002; Deuel et al., 2006; Kappeler et al., 2006; Koizumi et al., 2006; Nosten-Bertrand et al., 2008). DCX is expressed predominantly during brain development in migrating neurons, localizing particularly to the extremities of neuronal processes (Tint et al., 2009). Both DCX and DCLK1 bind and stabilise MTs, likely through a shared mechanism that prevents MT depolymerisation; *in vitro*, DCX also promotes MT nucleation independently of the nucleotide bound to tubulin (Bielas et al., 2007; Burgess and Reiner, 2000; Fourniol et al., 2010; Francis et al., 1999; Gleeson et al., 1999; Horesh et al., 1999; Lin et al., 2000; Moores et al., 2004; Moores et al., 2006; Vreugdenhil et al., 2007). The N-DC domain of DCX binds to the pockets between protofilaments at the corners of 4 αβ-tubulin dimers, enabling the selective stabilization of 13 protofilament MTs, which is the main MT architecture *in vivo* (Bechstedt and Brouhard, 2012; Fourniol et al., 2010; Liu et al., 2012; Moores et al., 2004; Tilney et al., 1973).

In addition to their roles in MT stabilisation, there is emerging evidence that DCX and DCLK are involved in regulation of Kif-dependent cargo distribution. Deuel and colleagues (2006) described phenotypes in double DCLK and DCX knockout mice that, in addition to stunted neuronal migration and axonal development, showed disrupted distribution of the synaptic vesicle proteins VAMP2 and synaptophysin. In dissociated hippocampal cultures of DCLK-/- neurons RNAi depleted of DCX, VAMP2 and synaptophysin failed to enter axons, instead being retained in the cell body, supporting a defect in synaptic vesicle distribution. Average axon length in either cultured DCLK-/- neurons, or WT neurons treated with DCX RNAi, is shorter than in wild-type non-transfected neurons, while DCLK-/- neurons with DCX RNAi have even shorter axons. As vesicle transport appears to be involved in neurite outgrowth and axonal growth cone-led neuronal migration (Kimura et al., 2003; Shirasu et al., 2000), this study suggested that, in addition to disruption of neuronal MTs themselves, human *DCX*-linked neuronal migration disorders could also result from perturbed cargo distribution.

As part of our efforts to characterise DCX-stabilised MTs *in vitro*, we used an ensemble MT gliding assay with Kif5 to compare the velocity of DCX-stabilized MTs with paclitaxel-stabilized GDP-MTs or GMPCPP-stabilized MTs. Robust motility was seen on all MT populations. However, a small (~16%) but significant reduction in velocity of DCX-MTs was observed (Moores et al., 2006). It is not clear, however, how this finding relates to the *in vivo* observations described above, since although Kif5 may carry some VAMP2-laden vesicles (Song et al., 2009), Kif1A appears primarily responsible for their transport (Liu et al., 2012; Okada and Hirokawa, 1999; Yonekawa et al., 1998). More recent work has further emphasised the differential effects by DCX/DCLK on kinesin motors and their cargo: in DCLK-/- deficient mouse neurons transfected with DCX shRNAi, Kif5 and its mitochondrial cargo show a normal distribution, while VAMP2 positive cargo is mislocalised within the soma (Liu et al., 2012). Time-lapse fluorescence imaging showed that this abnormal localization was coupled with shorter cargo run-lengths and a smaller number of mobile VAMP2 vesicles, although when mobile, they moved at wild-type velocities. No alterations in common tubulin PTMs or the localization of other MAPs were observed, and the abnormal transport and localization of Kif1A/VAMP2 could be rescued by transfection of a shRNAi-resistant WT - but not with lissencephaly-mutant containing - DCX constructs, verifying that DCX/DCLK are likely direct and specific regulators of Kif1A motors. The *in vivo* interaction of Kif1A and DCX was confirmed biochemically in fetal human brain and mouse brain, and in further support of this functional link, in cortical slice cultures Kif1A knockdown resulted in neuronal migration disruptions that phenocopy DCX/DCLK1 deficiency.

From a structural perspective, only cryo-EM reconstructions of DCX-MTs with and without bound Kif5 MD are currently available (Fourniol et al., 2010; Liu et al., 2012), but these structures already provide two major mechanistic insights: 1) the conformation of MT-bound DCX responds to the presence or absence of bound motor, specifically in the linkers on either side of N-DC the rest of the DCX molecule is not visible in these structures - and 2) there is direct interaction between these conformationally variable linkers within DCX and the bound motor, in loop regions of the motor domain which are different between Kif5 and Kif1A. Taken together, these data provide clear evidence for facilitation of Kif1A-cargo transport by DCX/DCLK, they suggest molecular mechanisms by which this could occur, and

highlight that in addition to abnormalities due to altered MT dynamics and organisation, DCX-linked pathologies may stem from cellular transport deficits.

Ensconsin/MAP7 promotes proper Kif5 distribution

In 2008 the *Drosophila* MAP ensconsin (mammalian MAP7 or E-MAP-115) was shown to be integral for Kif5 dependent processes in polarized oocytes and neurons, without being required for regulating overall MT architecture (Sung et al., 2008). Fly lines expressing ensconsin loss-of-function mutants exhibit abnormal oocyte development which was associated with improper distribution of Kif5, its *oskar* mRNA cargo and associated Staufen protein. In the neurons of these flies, abnormal ectopic distribution specifically of the Kif5 cargo synaptotagmin was also observed, diagnostic of its impaired anterograde transport. Using *in vitro* single motor assays in concentrated ovary extracts, the MT association rate and mobility of full length Kif5 were reduced in ensconsin mutant extracts; however, this was not the case for the truncated tail-less constitutively active Kif5 dimeric motor. This mechanism is under tight regulation by the MAP and ensconsin kinase PAR-1/MARK, indicating that further dynamic control of Kif5 can be achieved through alteration of ensconsin phosphorylation state.

More recently, using genetic mutants and RNA interference, ensconsin/MAP7 and Kif5B were identified as together being essential for proper nuclear positioning in *Drosophila* and cultured mammalian myotubes during muscle development (Metzger et al., 2012). The related MAPs MAP7D1, MAP7D2 or MAP7D3 on the other hand were not important in this process, indicating the specificity of this regulatory function of MAP7/ensconsin. Importantly, a physical interaction of the C-terminal coiled-coil region of MAP7 and the Kif5B tail was observed by co-immunoprecipitation, confirming previous evidence that MAP7/ensconsin-mediated regulation of Kif5 occurs at the motor tail. This work and a more recent study confirming the importance of ensconsin to Kif5 activation (Barlan et al., 2013) provide an enticing example of a MAP interacting with the tail domain, rather than motor domain, of Kif5. This interaction appears to control Kif5's specific recruitment, activation and thus its transport, likely through modulation of its auto-inhibitory capacity. In fact, Kif5 regulation can occur independently of ensconsin MT binding (i.e. its MAP activity). Further investigation of this interaction within a mammalian context is

warranted: although ensconsin/MAP7 may not function as a physiological regulator of MT dynamics, (Faire et al., 1999), its distribution is compartmentalized and somewhat polarized in neurons, and it is modulated by conditional phosphorylation (Bulinski et al., 2001; Sung et al., 2008), all of which are factors that are suggestive of a role for this MAP as a molecular signpost for Kifs. That this regulation may occur via the Kif5 tail also suggests that MAPs could also help manage motor activation and cargo loading to a specific Kif on a particular track.

Diseases associated with Kif distribution defects

Disrupted kinesin-based cargo transport is strongly implicated in the pathogenesis of a wide variety of conditions, including cancers, familial developmental abnormalities and various forms of neurodegenerative disease (De Vos et al., 2008; Liu et al., 2012; Yu and Feng, 2010). Whereas a number of conditions stem from familial mutations in the Kifs themselves - such as some hereditary spastic paraplegias (Kawaguchi, 2012; Klebe et al., 2012) – some of the remaining conditions may exhibit disrupted Kif-based transport due to perturbed MAP regulation of Kifs. Several of the most prominent examples are discussed below.

Alzheimer's Disease and the 'tauopathies'

Alzheimer's Disease (AD) is a prevalent neurodegenerative condition classically associated with high levels of aggregated Aβ peptides (resulting from mutations in the familial AD gene *APP* or unknown sporadic reasons) and abnormal phosphorylation, localization and aggregation of tau (Hardy, 2009; Iqbal et al., 2010; Small and Duff, 2008). Similar tau pathology, in the absence of Aβ pathology, is observed in the related 'tauopathies', in some cases due to mutations in the *MAPT* tau encoding gene itself (Hanger et al., 2009; Iqbal et al., 2010). In AD or tauopathy, transgenic mouse models or Aβ treated cells, disrupted axonal transport and an abnormal distribution of essential cargo such as mitochondria is observed, consistent with axonopathy seen in these model mice and post-mortem brain (De Vos et al., 2008; Stokin et al., 2005). Interestingly, AD models exhibit disrupted Kif5 mediated transport of APP perhaps contributing to the aberrant processing of APP that is associated with Aβ aggregation (Kamal et al., 2000; Lazarov et al., 2007; Stokin et al., 2005; Thinakaran and Koo, 2008).

Disruption to some of the regulatory mechanisms discussed above could contribute to transport defects in AD and related tauopathies. For example, there is strong evidence that in AD, tau becomes detached from MTs and as a result, is abnormally mislocalized and sequestered in hyperphosphorylated aggregates (Iqbal et al., 2010). Indeed, Aβ appears to cause the early phosphorylation of tau, causing MT dissociation (Amadoro et al., 2011; Reifert et al., 2011) and redistribution of tau from the axon to the somadendritic compartment in cultured neurons (Zempel et al., 2010). However, there is a continuing lack of consensus concerning the consequences of such tau redistribution for neuronal transport. Evidence that MT-bound tau at *physiological* concentrations facilitates Kif motility (Lopez and Sheetz, 1993; Peck et al., 2011; Seitz et al., 2002) suggests a 'loss of function' hypothesis for abnormal distribution of motors and cargo via tau hyperphosphorylation in AD affected neurons. Alternatively, high concentrations of tau on the MT - perhaps in an aggregated state - could inhibit Kif motility and perturb intracellular transport in AD (Ackmann et al., 2000; Baas and Qiang, 2005; Duan and Goodson, 2012; Mandelkow et al., 2003; Santarella et al., 2004). There is some evidence that total tau levels increase in AD, but this appears to correspond to an increase in clearance-resistant aggregated hyperphosphorylated forms, rather than an increase in newly produced soluble cytosolic tau, as judged by concentrations of mRNA (Hyman et al., 2005; Iqbal et al., 2010; Khatoon et al., 1992). Also in conflict with the 'roadblock' hypothesis: 1) tau extracted from AD brain has poor MT binding capacity and sequesters normal tau and other MAPs (Iqbal et al., 2008; Iqbal et al., 2010), 2) tau phosphorylation at sites detaching it from MTs appear critical for Aβ-mediated toxicity (Amadoro et al., 2011; Drewes, 2004; Iijima et al., 2010) and, 3) over-expression of tau appears not to affect axonal transport *in vivo* (Yuan et al., 2008). Furthermore, tau is in fact lost from axons and redistributed to the somato-dendritic compartment, implying that other, axonally-localised factors are responsible for the *reduced* axonal transport rates found in AD and the tauopathies (Ittner and Gotz, 2011; Kowall and Kosik, 1987; Zempel et al., 2010).

Despite these contradictions, it is possible that both 'loss of function' and 'roadblock' mechanisms could contribute to AD and the tauopathies, perhaps at different stages. For example, tau phosphorylation could be a protective response to remove an initial 'roadblock' insult caused by excessive tau MT binding. Eventually however, aggregation and

hyperphosphorylation of tau may cause its sequestration away from MTs and a 'loss of function' of Kif modulation. Other modulators of neuronal MTs and motors may subsequently be segregated in these aggregates: for example the human growth arrest-specific 7b protein (hGas7b) is highly expressed in brain tissues (Ju et al., 1998) and binds MTs, having independent and co-operative effects with tau on MT dynamics (Hidaka et al., 2012; Uchida et al., 2009). Interestingly, hGas7b binds to tau in a phosphorylation-dependent manner and there is a striking reduction in levels of this protein in AD brain (Akiyama et al., 2009) that could also contribute to abnormal motor/cargo transport and distribution. Even in the same cell, redistribution of tau could lead to simultaneous loss of function and aggregation in different compartments. In addition, transport dysfunction could be a consequence of disruption to the MT track itself due to tau's effects on MT dynamics and stability. Alternatively, transport may be affected by misplaced, aggregated and/or fragmented forms of tau independently of its MT binding as has been suggested, for example through activation of kinase pathways which act on Kifs (Kanaan et al., 2011), or as a downstream consequence of other speculated toxic 'gain-of-function' mechanisms of misplaced (Ittner and Gotz, 2011) or oligomeric tau species (Congdon and Duff, 2008).

Diseases related to DC domain containing proteins

Mutations in the X-linked *DCX* gene lead to subcortical band heterotopia or 'double cortex' syndrome in females, and lissencephaly or 'smooth brain' syndrome in males, associated with the abnormal migration of neurons leading to disrupted cortical layering and abnormal hippocampal development (des Portes et al., 1998a; des Portes et al., 1998b; Francis et al., 1999; Gleeson et al., 1998). As discussed, recent evidence has suggested that disease causing mutations result at least in part in the loss of function of DCX as a specific promoter of Kif1A transport, and improper distribution of its synaptic cargos (Deuel et al., 2006; Liu et al., 2012). That DCX-related disruptions to neuronal migration may be linked to improper Kif1A distribution is supported by the importance of DCX, Kif1A and vesicular synaptic cargos such as VAMP2 in neurite outgrowth and nuclear migration processes (Bai et al., 2003; Hepp and Langley, 2001; Kimura et al., 2003; Koizumi et al., 2006; Shirasu et al., 2000; Tsai et al., 2010). Interestingly, DCX deficiencies have recently been implicated in schizophrenia, which may also be associated with abnormal neuronal migration (Eastwood et al., 1995; Fung et al., 2011).

Mutations in the locus of another member of the DCX family, Retinitis Pigmentosa 1 (RP1), have been shown to account for roughly 5-10% of all autosomal dominant Retinitis Pigmentosas (Pagon and Daiger, 1993), a form of inherited blindness associated with retinal degeneration. RP1 contains N-terminal tandem DC domains much like DCX and associates with MTs and promotes their assembly and stability. However, it is expressed specifically in the retina where it associates with the MT-rich connecting cilium and outer segment axoneme of photoreceptors, promoting proper outer segment disc structure (Liu et al., 2003; Liu et al., 2002; Liu et al., 2004). It is possible that RP1 has some regulatory role on Kifs here but further work is needed to clarify this (Omori et al., 2010).

The gene locus for DCX family member doublecortin domain containing 2 (DCDC2), which contains tandem DC domains, has been linked to developmental dyslexia specifically affecting reading ability (Meng et al., 2005). Interestingly, dyslexia may result from a subtle defect in neuronal migration and DCDC2, in a manner somewhat similar to DCX, is important for this process in neuronal subpopulations migrating to brain regions important for reading ability (Burbridge et al., 2008; Galaburda et al., 2006; Meng et al., 2005). Importantly, DCDC2 co-localizes with MT rich structures such as the primary cilium in neurons, co-localizes and interacts with Kif3A, and modulates Wnt and Shh signalling possibly through its interaction with Kif3A (Massinen et al., 2011), however a direct effect of DCDC2 on Kif3A movement is yet to be demonstrated.

A 'MAP-kinesin code'?

Highly compartmentalized and/or polarized cells, such as neurons, require precise spatio-temporal regulation of motor associated cargo delivery to meet local needs. This could be achieved simply through diverse Kif motors and a variety of cargo-specific adaptor proteins as long as they are under the guidance of specific regulatory mechanisms. It is becoming clear that many selective, regulatory mechanisms exist, as exemplified recently by the demonstration in neurons that selective distribution of different Kifs is apparent at different developmental stages, and shows differential sensitivity to pharmacological treatments such as paclitaxel (Huang and Banker, 2011). In fact, it is likely that many regulatory mechanisms act in concert on a single Kif member to efficiently control both loading onto its MT tracks and its subsequent distribution.

Available evidence is currently overwhelmingly biased towards studies of Kif5-dependent transport in the axon. As mentioned above, some cargos appear to actively steer their own delivery to the dendritic compartment. Nevertheless - and as exemplified by Kif5 - some modulation may occur at the level of the MT itself, where elements in the Kif motor domain may recognize compartmentalized alterations in MT structure conferred by tubulin PTMs, or by tubulin isoform subunit composition. It is also apparent that modulation can occur through motor phosphorylation, cargo association, relief of Kif tail domain-mediated auto-inhibition (see Hirokawa et al., 2010; Verhey and Hammond, 2009) or possibly via direct involvement of motor tails in recognition of specific MTs (Seidel et al., 2012). However, these mechanisms are individually insufficient to account for all aspects of the often highly dynamic spatio-temporal regulation of Kifs.

The literature reviewed here demonstrates that neuronal MAPs make a contribution to the regulated distribution of some Kifs. This regulation may often be restricted to certain Kif family members, as in the case of DCX's specific regulation of Kif1A. Some MAPs may regulate the addition of particular tubulin PTMs to the MT. Some MAPs may alter MT architecture via their MT interaction, thereby allosterically altering the kinesin binding site and indirectly facilitating or hindering its binding and/or movement. Some MAPs may directly interfere with the MT binding of Kifs by competing with their binding sites, while others also trigger signalling pathways independently of MT binding to affect Kif behaviour. Interestingly, in some cases, MAPs may interact with the tail domain of Kifs (exemplified by ensconsin/MAP7), perhaps modulating the auto-inhibitory tail dependent mechanism of Kifs to alter their movement, a possibility often neglected by the common experimental utilisation of truncated constitutively active tail-less motors. The diverse modulatory effects of MAPs on specific Kif family members supports the existence of a 'MAP-Kif code' as suggested by Liu and colleagues (2012), that could work in conjunction with the covalent modifications associated with the 'tubulin code' (Verhey and Gaertig, 2007). The MAP code could provide dynamic spatio-temporal control of the distribution of Kifs and their cargo, and is perhaps disrupted in particular disease states. An important future topic of study of trafficking control is how polarised MAP distribution is initiated and maintained, and how it is coordinated with localisation of tubulin modifying enzymes. In addition, relatively little is known about how MAPs collaborate in transport control, with the apparently mutually

exclusive localisation of DCX and tau providing a particularly intriguing example (Tint et al., 2009). Many known MAPs have not yet been explicitly studied with reference to Kif regulation and with the regular addition of new MAPs to the existing repertoire, there may yet be many more MAPs shown to contribute to the code.

In fact, specific transport is likely the result of multiple interdependent factors including the MAP and tubulin codes and we are still rather far from a clear idea of how they are integrated. Important insights are yet to come to highlight the fundamental molecular mechanisms that allow these specific factors of the track to activate, recruit or regulate transport of not only kinesin but also dynein motors. Additional levels of coordination are also required with the actin tracks that frequently mediate local, short-range trafficking (e.g. Wagner et al., 2011) and some MAPs may be able to facilitate this MT-actin cross-talk (e.g. Fu et al., 2013). Progress in this field is critical to provide fundamental knowledge on the roadmaps that control trafficking processes that lie at the heart of cellular life.

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LEGENDS

Table 1. Comparison of neuronal human Kifs. The motor core sequence of human neuronal Kifs was compared with Kif5A (residue 8-325) using ClustalW (Larkin et al., 2007). Kifs expressed at moderate and high levels in neuronal cultures are included (relative abundance >1000 according to Silverman et al. (2010). Table references; [1]Charalambous et al. (2013), [2]Chu et al. (2006), [3]Guillaud et al. (2003), [4]Hammond et al. (2010), [5]Hanlon et al. (1997), [6]Hoang et al. (1999), [7]Huang and Banker (2011), [8]Jacobson et al. (2006)[9](Jenkins et al., 2012), [10]Kanai et al. (2000), [11]Kayadjanian et al. (2007), [12]Kondo et al. (1994), [13]Konishi and Setou (2009), [14]Lee et al. (2012), [15]Marszalek et al. (1999), [16]Mok et al. (2002), [17]Muresan et al. (1998), [18]Nakata and Hirokawa (2003), [19]Nakata et al. (2011), [20]Navone et al. (2001), [21]Noda et al. (2012), [22]Saito et al. (1997), [23]Setou et al. (2000), [24]Song et al. (2009), [25]Yamazaki et al. (1995), [26]Yang and Goldstein (1998)

Figure 1. N-Kif domain architecture and localisation of major tubulin PTMs. Schematic of a 13 protofilament MT, formed from αβ-tubulin heterodimers, showing the locations of common tubulin PTMs and dimeric kinesin-1 attachment. The lattice discontinuity called the seam is also indicated. β-tubulin can exist in GTP or GDP bound nucleotide states. Detyrosination of α-tubulin involves the removal of its extreme C-terminal Tyr present in most α-tubulin isotypes; this exposes a Glu at the C-terminus. Polyglutamylation is the addition of one or more additional Glu units to a Glu in the C-terminal tail of α and/or β-tubulin. Unlike other PTMs, acetylation of α-tubulin does not involve the tubulin C-terminal tail but most often occurs in the MT lumen on Lys40. Kinesin motor domains interact with individual αβ-tubulin heterodimers on the outside of the MT lattice. Below, a schematic of domain organization of a Kif5 dimer with attached cargo. The motor domain, coiled-coil stalk and tail region are encoded for by Kif family proteins, and either bind cargo directly, through associated kinesin light chain (KLC) subunits or other adaptor proteins (not shown).

Figure 2. Structural basis of Kif motor domain interaction with MTs. A) Crystal structure of human Kif5B (1BG2) with regions of sequence insertion and variation amongst neuronal

kinesins highlighted in green and red. These variations are found in the loop regions of the kinesin motor domain while its core structure is well conserved. Specific loop regions (L2, L8, L11) mentioned in the text are labeled in green. B) Front (top) and side (bottom) views of the kinesin-αβ-tubulin complex (PDB code 2P4N; Sindelar and Downing, 2007). Several of the loop variations are found at the motor-track interface and could thus account for differential motor-track specification. Others may be involved in modifying other aspects of motor function. Arrowheads indicate the location of the tubulin C-terminal tails. An arrow indicates the lumenal loop (disordered in this structure) containing the α-tubulin acetylation site α-40. ADP is shown in the Kif5B nucleotide binding pocket, the β-tubulin GTPase site contains GDP while the equivalent site in α-tubulin contains GTP. Figures were prepared using Chimera (Pettersen et al., 2004).

Figure 3. Domain structures of MAPs proposed to regulate kinesin transport. Schematic representation of human MAPs proposed to regulate kinesin distribution, divided into homologous subfamilies. Some MAPs, such as DCLK, tau and MAP2 exist as a number of alternatively spliced isoforms; in these cases the longest isoforms are shown. Nascent MAP1b is cleaved into heavy and light chains before recombining to form the full length protein. MAP7 is the mammalian homologue of *Drosophila* ensconsin. Abbreviations: MTBR; microtubule binding repeat (tau family). MTB; microtubule binding region (MAP1 family). EMTB; Ensconsin MT binding domain. Tau and doublecortin family proteins contain unrelated proline-rich regions that are major sites of serine/threonine phosphorylation.

Figure 4. Microtubule polarity, PTMs and MAPs vary within neuronal subdomains. A schematic representation of a polarized neuron indicating polarity of MTs, and PTM and MAP density within axons, dendrites and the soma. Axonal MTs are orientated nearly exclusively with +ends (red) distal and -ends (blue) proximal to the cell body, whereas somal and dendritic MTs have mixed polarity. GTP tubulin, Lys40 acetylation and detyrosination are found preferentially in the axonal domain, whereas polyglutamylation occurs throughout the neuron. Tau is found mainly in axons but is absent from the extreme axonal growth cone where DCX and DCLK are highly concentrated. MAP2 is found mainly in dendrites. DCX/DCLK and to a lesser extent MAP1b are found predominantly towards the extremities of growing processes, but also at lower levels throughout the rest of the neuron. There is currently insufficient evidence to attribute a clear sub-cellular distribution to ensconsin/MAP7.

References

*Ackmann, M., Wiech, H., and Mandelkow, E. (2000). Nonsaturable binding indicates clustering of tau on the microtubule surface in a paired helical filament-like conformation. J Biol Chem *275*, 30335-30343.

Adams, S.J., Crook, R.J., Deture, M., Randle, S.J., Innes, A.E., Yu, X.Z., Lin, W.L., Dugger, B.N., McBride, M., Hutton, M.*, et al.* (2009). Overexpression of wild-type murine tau results in progressive tauopathy and neurodegeneration. Am J Pathol *175*, 1598-1609.

Akhmanova, A., and Hammer, J.A., 3rd (2010). Linking molecular motors to membrane cargo. Curr Opin Cell Biol *22*, 479-487.

Akhmanova, A., and Steinmetz, M.O. (2008). Tracking the ends: a dynamic protein network controls the fate of microtubule tips. Nat Rev Mol Cell Biol *9*, 309-322.

Akiyama, H., Gotoh, A., Shin, R.W., Koga, T., Ohashi, T., Sakamoto, W., Harada, A., Arai, H., Sawa, A., Uchida, C.*, et al.* (2009). A novel role for hGas7b in microtubular maintenance: possible implication in tau-associated pathology in Alzheimer disease. J Biol Chem *284*, 32695-32699.

*Al-Bassam, J., Ozer, R.S., Safer, D., Halpain, S., and Milligan, R.A. (2002). MAP2 and tau bind longitudinally along the outer ridges of microtubule protofilaments. J Cell Biol *157*, 1187-1196.

Amadoro, G., Corsetti, V., Ciotti, M.T., Florenzano, F., Capsoni, S., Amato, G., and Calissano, P. (2011). Endogenous Abeta causes cell death via early tau hyperphosphorylation. Neurobiol Aging *32*, 969-990.

Amos, L.A., and Schlieper, D. (2005). Microtubules and maps. Adv Protein Chem *71*, 257-298.

Audebert, S., Koulakoff, A., Berwald-Netter, Y., Gros, F., Denoulet, P., and Edde, B. (1994). Developmental regulation of polyglutamylated alpha- and beta-tubulin in mouse brain neurons. J Cell Sci *107 (Pt 8)*, 2313-2322.

*Baas, P.W., Deitch, J.S., Black, M.M., and Banker, G.A. (1988). Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc Natl Acad Sci U S A *85*, 8335-8339.

Baas, P.W., and Qiang, L. (2005). Neuronal microtubules: when the MAP is the roadblock. Trends Cell Biol *15*, 183-187.

Bai, J., Ramos, R.L., Ackman, J.B., Thomas, A.M., Lee, R.V., and LoTurco, J.J. (2003). RNAi reveals doublecortin is required for radial migration in rat neocortex. Nat Neurosci *6*, 1277-1283.

Bananis, E., Nath, S., Gordon, K., Satir, P., Stockert, R.J., Murray, J.W., and Wolkoff, A.W. (2004). Microtubule-dependent movement of late endocytic vesicles in vitro: requirements for Dynein and Kinesin. Mol Biol Cell *15*, 3688-3697.

Barlan, K., Lu, W., and Gelfand, V.I. (2013). The Microtubule-Binding Protein Ensconsin Is an Essential Cofactor of Kinesin-1. Curr Biol.

Bechstedt, S., and Brouhard, G.J. (2012). Doublecortin recognizes the 13-protofilament microtubule cooperatively and tracks microtubule ends. Dev Cell *23*, 181-192.

Bielas, S.L., Serneo, F.F., Chechlacz, M., Deerinck, T.J., Perkins, G.A., Allen, P.B., Ellisman, M.H., and Gleeson, J.G. (2007). Spinophilin facilitates dephosphorylation of doublecortin by PP1 to mediate microtubule bundling at the axonal wrist. Cell *129*, 579-591.

Bieling, P., Telley, I.A., and Surrey, T. (2010). A minimal midzone protein module controls formation and length of antiparallel microtubule overlaps. Cell *142*, 420-432.

Binder, L.I., Frankfurter, A., and Rebhun, L.I. (1985). The distribution of tau in the mammalian central nervous system. J Cell Biol *101*, 1371-1378.

Bobinnec, Y., Moudjou, M., Fouquet, J.P., Desbruyeres, E., Edde, B., and Bornens, M. (1998). Glutamylation of centriole and cytoplasmic tubulin in proliferating non-neuronal cells. Cell Motil Cytoskeleton *39*, 223-232.

Bonnet, C., Boucher, D., Lazereg, S., Pedrotti, B., Islam, K., Denoulet, P., and Larcher, J.C. (2001). Differential binding regulation of microtubule-associated proteins MAP1A, MAP1B, and MAP2 by tubulin polyglutamylation. J Biol Chem *276*, 12839-12848.

Boucher, D., Larcher, J.C., Gros, F., and Denoulet, P. (1994). Polyglutamylation of tubulin as a progressive regulator of in vitro interactions between the microtubule-associated protein Tau and tubulin. Biochemistry *33*, 12471-12477.

Boyne, L.J., Tessler, A., Murray, M., and Fischer, I. (1995). Distribution of Big tau in the central nervous system of the adult and developing rat. J Comp Neurol *358*, 279-293.

Bulinski, J.C., McGraw, T.E., Gruber, D., Nguyen, H.L., and Sheetz, M.P. (1997). Overexpression of MAP4 inhibits organelle motility and trafficking in vivo. J Cell Sci *110 (Pt 24)*, 3055-3064.

Bulinski, J.C., Odde, D.J., Howell, B.J., Salmon, T.D., and Waterman-Storer, C.M. (2001). Rapid dynamics of the microtubule binding of ensconsin in vivo. J Cell Sci *114*, 3885-3897.

Burbridge, T.J., Wang, Y., Volz, A.J., Peschansky, V.J., Lisann, L., Galaburda, A.M., Lo Turco, J.J., and Rosen, G.D. (2008). Postnatal analysis of the effect of embryonic knockdown and overexpression of candidate dyslexia susceptibility gene homolog Dcdc2 in the rat. Neuroscience *152*, 723-733.

Burgess, H.A., and Reiner, O. (2000). Doublecortin-like kinase is associated with microtubules in neuronal growth cones. Mol Cell Neurosci *16*, 529-541.

Byrd, D.T., Kawasaki, M., Walcoff, M., Hisamoto, N., Matsumoto, K., and Jin, Y. (2001). UNC-16, a JNK-signaling scaffold protein, regulates vesicle transport in C. elegans. Neuron *32*, 787-800.

Cai, D., McEwen, D.P., Martens, J.R., Meyhofer, E., and Verhey, K.J. (2009). Single molecule imaging reveals differences in microtubule track selection between Kinesin motors. PLoS Biol *7*, e1000216.

Charalambous, D.C., Pasciuto, E., Mercaldo, V., Pilo Boyl, P., Munck, S., Bagni, C., and Santama, N. (2013). KIF1Bbeta transports dendritically localized mRNPs in neurons and is recruited to synapses in an activity-dependent manner. Cell Mol Life Sci *70*, 335-356.

Chu, P.J., Rivera, J.F., and Arnold, D.B. (2006). A role for Kif17 in transport of Kv4.2. J Biol Chem *281*, 365-373.

Cierpicki, T., Kim, M.H., Cooper, D.R., Derewenda, U., Bushweller, J.H., and Derewenda, Z.S. (2006). The DC-module of doublecortin: dynamics, domain boundaries, and functional implications. Proteins *64*, 874-882.

Congdon, E.E., and Duff, K.E. (2008). Is tau aggregation toxic or protective? J Alzheimers Dis *14*, 453-457.

Cook, C., Gendron, T.F., Scheffel, K., Carlomagno, Y., Dunmore, J., DeTure, M., and Petrucelli, L. (2012). Loss of HDAC6, a novel CHIP substrate, alleviates abnormal tau accumulation. Hum Mol Genet *21*, 2936-2945.

Corbo, J.C., Deuel, T.A., Long, J.M., LaPorte, P., Tsai, E., Wynshaw-Boris, A., and Walsh, C.A. (2002). Doublecortin is required in mice for lamination of the hippocampus but not the neocortex. J Neurosci *22*, 7548-7557.

De Vos, K.J., Grierson, A.J., Ackerley, S., and Miller, C.C. (2008). Role of axonal transport in neurodegenerative diseases. Annu Rev Neurosci *31*, 151-173.

des Portes, V., Francis, F., Pinard, J.M., Desguerre, I., Moutard, M.L., Snoeck, I., Meiners, L.C., Capron, F., Cusmai, R., Ricci, S.*, et al.* (1998a). doublecortin is the major gene causing X-linked subcortical laminar heterotopia (SCLH). Hum Mol Genet *7*, 1063-1070.

des Portes, V., Pinard, J.M., Billuart, P., Vinet, M.C., Koulakoff, A., Carrie, A., Gelot, A., Dupuis, E., Motte, J., Berwald-Netter, Y.*, et al.* (1998b). A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. Cell *92*, 51-61.

Desai, A., and Mitchison, T.J. (1997). Microtubule polymerization dynamics. Annu Rev Cell Dev Biol *13*, 83-117.

Deshpande, A., Win, K.M., and Busciglio, J. (2008). Tau isoform expression and regulation in human cortical neurons. FASEB J *22*, 2357-2367.

*Deuel, T.A., Liu, J.S., Corbo, J.C., Yoo, S.Y., Rorke-Adams, L.B., and Walsh, C.A. (2006). Genetic interactions between doublecortin and doublecortin-like kinase in neuronal migration and axon outgrowth. Neuron *49*, 41-53.

Dijkmans, T.F., van Hooijdonk, L.W., Fitzsimons, C.P., and Vreugdenhil, E. (2010). The doublecortin gene family and disorders of neuronal structure. Cent Nerv Syst Agents Med Chem *10*, 32-46.

*Dimitrov, A., Quesnoit, M., Moutel, S., Cantaloube, I., Pous, C., and Perez, F. (2008). Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues. Science *322*, 1353-1356.

DiTella, M.C., Feiguin, F., Carri, N., Kosik, K.S., and Caceres, A. (1996). MAP-1B/TAU functional redundancy during laminin-enhanced axonal growth. J Cell Sci *109 (Pt 2)*, 467-477.

*Dixit, R., Ross, J.L., Goldman, Y.E., and Holzbaur, E.L. (2008). Differential regulation of dynein and kinesin motor proteins by tau. Science *319*, 1086-1089.

Douglas, M.E., and Mishima, M. (2010). Still entangled: assembly of the central spindle by multiple microtubule modulators. Semin Cell Dev Biol *21*, 899-908.

Drewes, G. (2004). MARKing tau for tangles and toxicity. Trends Biochem Sci *29*, 548-555.

Drewes, G., Ebneth, A., and Mandelkow, E.M. (1998). MAPs, MARKs and microtubule dynamics. Trends Biochem Sci *23*, 307-311.

Duan, A.R., and Goodson, H.V. (2012). Taxol-stabilized microtubules promote the formation of filaments from unmodified full-length Tau in vitro. Mol Biol Cell *23*, 4796-4806.

Dubey, M., Chaudhury, P., Kabiru, H., and Shea, T.B. (2008). Tau inhibits anterograde axonal transport and perturbs stability in growing axonal neurites in part by displacing kinesin cargo: neurofilaments attenuate tau-mediated neurite instability. Cell Motil Cytoskeleton *65*, 89-99.

Duff, K., and Suleman, F. (2004). Transgenic mouse models of Alzheimer's disease: how useful have they been for therapeutic development? Brief Funct Genomic Proteomic *3*, 47-59.

Dunn, S., Morrison, E.E., Liverpool, T.B., Molina-Paris, C., Cross, R.A., Alonso, M.C., and Peckham, M. (2008). Differential trafficking of Kif5c on tyrosinated and detyrosinated microtubules in live cells. J Cell Sci *121*, 1085-1095.

Eastwood, S.L., Burnet, P.W., and Harrison, P.J. (1995). Altered synaptophysin expression as a marker of synaptic pathology in schizophrenia. Neuroscience *66*, 309-319.

Ebneth, A., Godemann, R., Stamer, K., Illenberger, S., Trinczek, B., and Mandelkow, E. (1998). Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. J Cell Biol *143*, 777-794.

Erck, C., Peris, L., Andrieux, A., Meissirel, C., Gruber, A.D., Vernet, M., Schweitzer, A., Saoudi, Y., Pointu, H., Bosc, C.*, et al.* (2005). A vital role of tubulin-tyrosine-ligase for neuronal organization. Proc Natl Acad Sci U S A *102*, 7853-7858.

Faire, K., Waterman-Storer, C.M., Gruber, D., Masson, D., Salmon, E.D., and Bulinski, J.C. (1999). E-MAP-115 (ensconsin) associates dynamically with microtubules in vivo and is not a physiological modulator of microtubule dynamics. J Cell Sci *112 (Pt 23)*, 4243-4255.

Fourniol, F.J., Sindelar, C.V., Amigues, B., Clare, D.K., Thomas, G., Perderiset, M., Francis, F., Houdusse, A., and Moores, C.A. (2010). Template-free 13-protofilament microtubule-MAP assembly visualized at 8 A resolution. J Cell Biol *191*, 463-470.

Francis, F., Koulakoff, A., Boucher, D., Chafey, P., Schaar, B., Vinet, M.C., Friocourt, G., McDonnell, N., Reiner, O., Kahn, A.*, et al.* (1999). Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. Neuron *23*, 247-256.

Fu, X., Brown, K.J., Yap, C.C., Winckler, B., Jaiswal, J.K., and Liu, J.S. (2013). Doublecortin (dcx) family proteins regulate filamentous actin structure in developing neurons. J Neurosci *33*, 709-721.

Fukushima, N., Furuta, D., Hidaka, Y., Moriyama, R., and Tsujiuchi, T. (2009). Post-translational modifications of tubulin in the nervous system. J Neurochem *109*, 683-693.

Fung, S.J., Joshi, D., Allen, K.M., Sivagnanasundaram, S., Rothmond, D.A., Saunders, R., Noble, P.L., Webster, M.J., and Weickert, C.S. (2011). Developmental patterns of doublecortin expression and white matter neuron density in the postnatal primate prefrontal cortex and schizophrenia. PLoS One *6*, e25194.

Galaburda, A.M., LoTurco, J., Ramus, F., Fitch, R.H., and Rosen, G.D. (2006). From genes to behavior in developmental dyslexia. Nat Neurosci *9*, 1213-1217.

Garnham, C.P., and Roll-Mecak, A. (2012). The chemical complexity of cellular microtubules: tubulin post-translational modification enzymes and their roles in tuning microtubule functions. Cytoskeleton (Hoboken) *69*, 442-463.

Gleeson, J.G., Allen, K.M., Fox, J.W., Lamperti, E.D., Berkovic, S., Scheffer, I., Cooper, E.C., Dobyns, W.B., Minnerath, S.R., Ross, M.E.*, et al.* (1998). Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell *92*, 63-72.

Gleeson, J.G., Lin, P.T., Flanagan, L.A., and Walsh, C.A. (1999). Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. Neuron *23*, 257-271.

Guillaud, L., Setou, M., and Hirokawa, N. (2003). KIF17 dynamics and regulation of NR2B trafficking in hippocampal neurons. J Neurosci *23*, 131-140.

Gurland, G., and Gundersen, G.G. (1995). Stable, detyrosinated microtubules function to localize vimentin intermediate filaments in fibroblasts. J Cell Biol *131*, 1275-1290.

Hagiwara, H., Yorifuji, H., Sato-Yoshitake, R., and Hirokawa, N. (1994). Competition between motor molecules (kinesin and cytoplasmic dynein) and fibrous microtubule-associated proteins in binding to microtubules. J Biol Chem *269*, 3581-3589.

*Hammond, J.W., Huang, C.F., Kaech, S., Jacobson, C., Banker, G., and Verhey, K.J. (2010). Posttranslational modifications of tubulin and the polarized transport of kinesin-1 in neurons. Mol Biol Cell *21*, 572-583.

Hanger, D.P., Anderton, B.H., and Noble, W. (2009). Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. Trends Mol Med *15*, 112-119.

Hanlon, D.W., Yang, Z., and Goldstein, L.S. (1997). Characterization of KIFC2, a neuronal kinesin superfamily member in mouse. Neuron *18*, 439-451.

Harada, A., Oguchi, K., Okabe, S., Kuno, J., Terada, S., Ohshima, T., Sato-Yoshitake, R., Takei, Y., Noda, T., and Hirokawa, N. (1994). Altered microtubule organization in small-calibre axons of mice lacking tau protein. Nature *369*, 488-491.

Hardy, J. (2009). The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. J Neurochem *110*, 1129-1134.

*Heins, S., Song, Y.H., Wille, H., Mandelkow, E., and Mandelkow, E.M. (1991). Effect of MAP2, MAP2c, and tau on kinesin-dependent microtubule motility. J Cell Sci Suppl *14*, 121-124.

Hepp, R., and Langley, K. (2001). SNAREs during development. Cell Tissue Res *305*, 247-253.

Hidaka, M., Koga, T., Gotoh, A., Sanada, M., Hirose, K., and Uchida, T. (2012). Alzheimer's disease-related protein hGas7b interferes with kinesin motility. J Biochem *151*, 593-598.

Hirokawa, N., Niwa, S., and Tanaka, Y. (2010). Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. Neuron *68*, 610-638.

Hirokawa, N., Noda, Y., Tanaka, Y., and Niwa, S. (2009). Kinesin superfamily motor proteins and intracellular transport. Nat Rev Mol Cell Biol *10*, 682-696.

Hoang, E., Bost-Usinger, L., and Burnside, B. (1999). Characterization of a novel C-kinesin (KIFC3) abundantly expressed in vertebrate retina and RPE. Exp Eye Res *69*, 57-68.

Horesh, D., Sapir, T., Francis, F., Wolf, S.G., Caspi, M., Elbaum, M., Chelly, J., and Reiner, O. (1999). Doublecortin, a stabilizer of microtubules. Hum Mol Genet *8*, 1599-1610.

Horiguchi, K., Hanada, T., Fukui, Y., and Chishti, A.H. (2006). Transport of PIP3 by GAKIN, a kinesin-3 family protein, regulates neuronal cell polarity. J Cell Biol *174*, 425-436.

Huang, C.F., and Banker, G. (2011). The Translocation Selectivity of the Kinesins that Mediate Neuronal Organelle Transport. Traffic. Traffic *13*, 549-564.

Hyman, B.T., Augustinack, J.C., and Ingelsson, M. (2005). Transcriptional and conformational changes of the tau molecule in Alzheimer's disease. Biochim Biophys Acta *1739*, 150-157.

Iijima, K., Gatt, A., and Iijima-Ando, K. (2010). Tau Ser262 phosphorylation is critical for Abeta42-induced tau toxicity in a transgenic Drosophila model of Alzheimer's disease. Hum Mol Genet *19*, 2947-2957.

Ikegami, K., Heier, R.L., Taruishi, M., Takagi, H., Mukai, M., Shimma, S., Taira, S., Hatanaka, K., Morone, N., Yao, I., et al.* (2007). Loss of alpha-tubulin polyglutamylation in ROSA22 mice is associated with abnormal targeting of KIF1A and modulated synaptic function. Proc Natl Acad Sci U S A *104*, 3213-3218.

Ikegami, K., Mukai, M., Tsuchida, J., Heier, R.L., Macgregor, G.R., and Setou, M. (2006). TTLL7 is a mammalian beta-tubulin polyglutamylase required for growth of MAP2-positive neurites. J Biol Chem *281*, 30707-30716.

Iqbal, K., Alonso Adel, C., and Grundke-Iqbal, I. (2008). Cytosolic abnormally hyperphosphorylated tau but not paired helical filaments sequester normal MAPs and inhibit microtubule assembly. J Alzheimers Dis *14*, 365-370.

Iqbal, K., Liu, F., Gong, C.X., and Grundke-Iqbal, I. (2010). Tau in Alzheimer disease and related tauopathies. Curr Alzheimer Res *7*, 656-664.

Ittner, L.M., and Gotz, J. (2011). Amyloid-beta and tau--a toxic pas de deux in Alzheimer's disease. Nat Rev Neurosci *12*, 65-72.

*Jacobson, C., Schnapp, B., and Banker, G.A. (2006). A change in the selective translocation of the Kinesin-1 motor domain marks the initial specification of the axon. Neuron *49*, 797-804.

Janke, C., and Bulinski, J.C. (2011). Post-translational regulation of the microtubule cytoskeleton: mechanisms and functions. Nat Rev Mol Cell Biol *12*, 773-786.

Jenkins, B., Decker, H., Bentley, M., Luisi, J., and Banker, G. (2012). A novel split kinesin assay identifies motor proteins that interact with distinct vesicle populations. J Cell Biol *198*, 749-761.

Jimenez-Mateos, E.M., Gonzalez-Billault, C., Dawson, H.N., Vitek, M.P., and Avila, J. (2006). Role of MAP1B in axonal retrograde transport of mitochondria. Biochem J *397*, 53-59.

Ju, Y.T., Chang, A.C., She, B.R., Tsaur, M.L., Hwang, H.M., Chao, C.C., Cohen, S.N., and Lin-Chao, S. (1998). gas7: A gene expressed preferentially in growth-arrested fibroblasts and terminally differentiated Purkinje neurons affects neurite formation. Proc Natl Acad Sci U S A *95*, 11423-11428.

Kamal, A., Stokin, G.B., Yang, Z., Xia, C.H., and Goldstein, L.S. (2000). Axonal transport of amyloid precursor protein is mediated by direct binding to the kinesin light chain subunit of kinesin-I. Neuron *28*, 449-459.

*Kanaan, N.M., Morfini, G.A., LaPointe, N.E., Pigino, G.F., Patterson, K.R., Song, Y., Andreadis, A., Fu, Y., Brady, S.T., and Binder, L.I. (2011). Pathogenic forms of tau inhibit kinesin-dependent axonal transport through a mechanism involving activation of axonal phosphotransferases. J Neurosci *31*, 9858-9868.

Kanai, Y., Okada, Y., Tanaka, Y., Harada, A., Terada, S., and Hirokawa, N. (2000). KIF5C, a novel neuronal kinesin enriched in motor neurons. J Neurosci *20*, 6374-6384.

Kappeler, C., Saillour, Y., Baudoin, J.P., Tuy, F.P., Alvarez, C., Houbron, C., Gaspar, P., Hamard, G., Chelly, J., Metin, C.*, et al.* (2006). Branching and nucleokinesis defects in migrating interneurons derived from doublecortin knockout mice. Hum Mol Genet *15*, 1387-1400.

*Kar, S., Fan, J., Smith, M.J., Goedert, M., and Amos, L.A. (2003). Repeat motifs of tau bind to the insides of microtubules in the absence of taxol. EMBO J *22*, 70-77.

Kaslow, H.R., Groppi, V.E., Abood, M.E., and Bourne, H.R. (1981). Cholera toxin can catalyze ADP-ribosylation of cytoskeletal proteins. J Cell Biol *91*, 410-413.

Kawaguchi, K. (2012). Role of Kinesin-1 in the Pathogenesis of SPG10, a Rare Form of Hereditary Spastic Paraplegia. Neuroscientist.

Kayadjanian, N., Lee, H.S., Pina-Crespo, J., and Heinemann, S.F. (2007). Localization of glutamate receptors to distal dendrites depends on subunit composition and the kinesin motor protein KIF17. Mol Cell Neurosci *34*, 219-230.

Khatoon, S., Grundke-Iqbal, I., and Iqbal, K. (1992). Brain levels of microtubule-associated protein tau are elevated in Alzheimer's disease: a radioimmuno-slot-blot assay for nanograms of the protein. J Neurochem *59*, 750-753.

Khawaja, S., Gundersen, G.G., and Bulinski, J.C. (1988). Enhanced stability of microtubules enriched in detyrosinated tubulin is not a direct function of detyrosination level. J Cell Biol *106*, 141-149.

Kim, M.H., Cierpicki, T., Derewenda, U., Krowarsch, D., Feng, Y., Devedjiev, Y., Dauter, Z., Walsh, C.A., Otlewski, J., Bushweller, J.H.*, et al.* (2003). The DCX-domain tandems of doublecortin and doublecortin-like kinase. Nat Struct Biol *10*, 324-333.

Kimura, K., Mizoguchi, A., and Ide, C. (2003). Regulation of growth cone extension by SNARE proteins. J Histochem Cytochem *51*, 429-433.

Klebe, S., Lossos, A., Azzedine, H., Mundwiller, E., Sheffer, R., Gaussen, M., Marelli, C., Nawara, M., Carpentier, W., Meyer, V.*, et al.* (2012). KIF1A missense mutations in SPG30, an autosomal recessive spastic paraplegia: distinct phenotypes according to the nature of the mutations. Eur J Hum Genet *20*, 645-649.

Koizumi, H., Tanaka, T., and Gleeson, J.G. (2006). Doublecortin-like kinase functions with doublecortin to mediate fiber tract decussation and neuronal migration. Neuron *49*, 55-66.

Kondo, S., Sato-Yoshitake, R., Noda, Y., Aizawa, H., Nakata, T., Matsuura, Y., and Hirokawa, N. (1994). KIF3A is a new microtubule-based anterograde motor in the nerve axon. J Cell Biol *125*, 1095-1107.

*Konishi, Y., and Setou, M. (2009). Tubulin tyrosination navigates the kinesin-1 motor domain to axons. Nat Neurosci *12*, 559-567.

Kowall, N.W., and Kosik, K.S. (1987). Axonal disruption and aberrant localization of tau protein characterize the neuropil pathology of Alzheimer's disease. Ann Neurol *22*, 639-643.

Kreitzer, G., Liao, G., and Gundersen, G.G. (1999). Detyrosination of tubulin regulates the interaction of intermediate filaments with microtubules in vivo via a kinesin-dependent mechanism. Mol Biol Cell *10*, 1105-1118.

Kueh, H.Y., and Mitchison, T.J. (2009). Structural plasticity in actin and tubulin polymer dynamics. Science *325*, 960-963.

LaPointe, N.E., Morfini, G., Pigino, G., Gaisina, I.N., Kozikowski, A.P., Binder, L.I., and Brady, S.T. (2009). The amino terminus of tau inhibits kinesin-dependent axonal transport: implications for filament toxicity. J Neurosci Res *87*, 440-451.

Larcher, J.C., Boucher, D., Lazereg, S., Gros, F., and Denoulet, P. (1996). Interaction of kinesin motor domains with alpha- and beta-tubulin subunits at a tau-independent binding site. Regulation by polyglutamylation. J Biol Chem *271*, 22117-22124.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R.*, et al.* (2007). Clustal W and Clustal X version 2.0. Bioinformatics *23*, 2947-2948.

Lazarov, O., Morfini, G.A., Pigino, G., Gadadhar, A., Chen, X., Robinson, J., Ho, H., Brady, S.T., and Sisodia, S.S. (2007). Impairments in fast axonal transport and motor neuron deficits in transgenic mice expressing familial Alzheimer's disease-linked mutant presenilin 1. J Neurosci *27*, 7011-7020.

Lee, K.H., Lee, J.S., Lee, D., Seog, D.H., Lytton, J., Ho, W.K., and Lee, S.H. (2012). KIF21A-mediated axonal transport and selective endocytosis underlie the polarized targeting of NCKX2. J Neurosci *32*, 4102-4117.

Liao, G., and Gundersen, G.G. (1998). Kinesin is a candidate for cross-bridging microtubules and intermediate filaments. Selective binding of kinesin to detyrosinated tubulin and vimentin. J Biol Chem *273*, 9797-9803.

Lin, P.T., Gleeson, J.G., Corbo, J.C., Flanagan, L., and Walsh, C.A. (2000). DCAMKL1 encodes a protein kinase with homology to doublecortin that regulates microtubule polymerization. J Neurosci *20*, 9152-9161.

*Liu, J.S., Schubert, C.R., Fu, X., Fourniol, F.J., Jaiswal, J.K., Houdusse, A., Stultz, C.M., Moores, C.A., and Walsh, C.A. (2012). Molecular basis for specific regulation of neuronal kinesin-3 motors by doublecortin family proteins. Mol Cell *47*, 707-721.

Liu, Q., Lyubarsky, A., Skalet, J.H., Pugh, E.N., Jr., and Pierce, E.A. (2003). RP1 is required for the correct stacking of outer segment discs. Invest Ophthalmol Vis Sci *44*, 4171-4183.

Liu, Q., Zhou, J., Daiger, S.P., Farber, D.B., Heckenlively, J.R., Smith, J.E., Sullivan, L.S., Zuo, J., Milam, A.H., and Pierce, E.A. (2002). Identification and subcellular localization of the RP1 protein in human and mouse photoreceptors. Invest Ophthalmol Vis Sci *43*, 22-32.

Liu, Q., Zuo, J., and Pierce, E.A. (2004). The retinitis pigmentosa 1 protein is a photoreceptor microtubule-associated protein. J Neurosci *24*, 6427-6436.

*Lopez, L.A., and Sheetz, M.P. (1993). Steric inhibition of cytoplasmic dynein and kinesin motility by MAP2. Cell Motil Cytoskeleton *24*, 1-16.

Maas, C., Belgardt, D., Lee, H.K., Heisler, F.F., Lappe-Siefke, C., Magiera, M.M., van Dijk, J., Hausrat, T.J., Janke, C., and Kneussel, M. (2009). Synaptic activation modifies microtubules underlying transport of postsynaptic cargo. Proc Natl Acad Sci U S A *106*, 8731-8736.

*Makrides, V., Massie, M.R., Feinstein, S.C., and Lew, J. (2004). Evidence for two distinct binding sites for tau on microtubules. Proc Natl Acad Sci U S A *101*, 6746-6751.

*Mandelkow, E.M., Stamer, K., Vogel, R., Thies, E., and Mandelkow, E. (2003). Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. Neurobiol Aging *24*, 1079-1085.

Mandelkow, E.M., Thies, E., Trinczek, B., Biernat, J., and Mandelkow, E. (2004). MARK/PAR1 kinase is a regulator of microtubule-dependent transport in axons. J Cell Biol *167*, 99-110.

Mandell, J.W., and Banker, G.A. (1996). A spatial gradient of tau protein phosphorylation in nascent axons. J Neurosci *16*, 5727-5740.

Marcos, S., Moreau, J., Backer, S., Job, D., Andrieux, A., and Bloch-Gallego, E. (2009). Tubulin tyrosination is required for the proper organization and pathfinding of the growth cone. PLoS One *4*, e5405.

Marszalek, J.R., Weiner, J.A., Farlow, S.J., Chun, J., and Goldstein, L.S. (1999). Novel dendritic kinesin sorting identified by different process targeting of two related kinesins: KIF21A and KIF21B. J Cell Biol *145*, 469-479.

Massinen, S., Hokkanen, M.E., Matsson, H., Tammimies, K., Tapia-Paez, I., Dahlstrom-Heuser, V., Kuja-Panula, J., Burghoorn, J., Jeppsson, K.E., Swoboda, P.*, et al.* (2011). Increased expression of the dyslexia candidate gene DCDC2 affects length and signaling of primary cilia in neurons. PLoS One *6*, e20580.

Mattie, F.J., Stackpole, M.M., Stone, M.C., Clippard, J.R., Rudnick, D.A., Qiu, Y., Tao, J., Allender, D.L., Parmar, M., and Rolls, M.M. (2010). Directed microtubule growth, +TIPs, and kinesin-2 are required for uniform microtubule polarity in dendrites. Curr Biol *20*, 2169-2177.

Maurer, S.P., Bieling, P., Cope, J., Hoenger, A., and Surrey, T. (2011). GTPgammaS microtubules mimic the growing microtubule end structure recognized by end-binding proteins (EBs). Proc Natl Acad Sci U S A *108*, 3988-3993.

Maurer, S.P., Fourniol, F.J., Bohner, G., Moores, C.A., and Surrey, T. (2012). EBs recognize a nucleotide-dependent structural cap at growing microtubule ends. Cell *149*, 371-382.

*McVicker, D.P., Chrin, L.R., and Berger, C.L. (2011). The nucleotide-binding state of microtubules modulates kinesin processivity and the ability of Tau to inhibit kinesin-mediated transport. J Biol Chem *286*, 42873-42880.

Meng, H., Smith, S.D., Hager, K., Held, M., Liu, J., Olson, R.K., Pennington, B.F., DeFries, J.C., Gelernter, J., O'Reilly-Pol, T.*, et al.* (2005). DCDC2 is associated with reading disability and modulates neuronal development in the brain. Proc Natl Acad Sci U S A *102*, 17053-17058.

Metzger, T., Gache, V., Xu, M., Cadot, B., Folker, E.S., Richardson, B.E., Gomes, E.R., and Baylies, M.K. (2012). MAP and kinesin-dependent nuclear positioning is required for skeletal muscle function. Nature *484*, 120-124.

Miki, H., Setou, M., and Hirokawa, N. (2003). Kinesin superfamily proteins (KIFs) in the mouse transcriptome. Genome Res *13*, 1455-1465.

Mok, H., Shin, H., Kim, S., Lee, J.R., Yoon, J., and Kim, E. (2002). Association of the kinesin superfamily motor protein KIF1Balpha with postsynaptic density-95 (PSD-95), synapse-associated protein-97, and synaptic scaffolding molecule PSD-95/discs large/zona occludens-1 proteins. J Neurosci *22*, 5253-5258.

Montenegro Gouveia, S., Leslie, K., Kapitein, L.C., Buey, R.M., Grigoriev, I., Wagenbach, M., Smal, I., Meijering, E., Hoogenraad, C.C., Wordeman, L.*, et al.* (2010). In vitro reconstitution of the functional interplay between MCAK and EB3 at microtubule plus ends. Curr Biol *20*, 1717-1722.

Moores, C.A., Perderiset, M., Francis, F., Chelly, J., Houdusse, A., and Milligan, R.A. (2004). Mechanism of microtubule stabilization by doublecortin. Mol Cell *14*, 833-839.

Moores, C.A., Perderiset, M., Kappeler, C., Kain, S., Drummond, D., Perkins, S.J., Chelly, J., Cross, R., Houdusse, A., and Francis, F. (2006). Distinct roles of doublecortin modulating the microtubule cytoskeleton. EMBO J *25*, 4448-4457.

Morfini, G., Pigino, G., Mizuno, N., Kikkawa, M., and Brady, S.T. (2007). Tau binding to microtubules does not directly affect microtubule-based vesicle motility. J Neurosci Res *85*, 2620-2630.

Muresan, V., Abramson, T., Lyass, A., Winter, D., Porro, E., Hong, F., Chamberlin, N.L., and Schnapp, B.J. (1998). KIF3C and KIF3A form a novel neuronal heteromeric kinesin that associates with membrane vesicles. Mol Biol Cell *9*, 637-652.

Nadar, V.C., Ketschek, A., Myers, K.A., Gallo, G., and Baas, P.W. (2008). Kinesin-5 is essential for growth-cone turning. Curr Biol *18*, 1972-1977.

*Nakata, T., and Hirokawa, N. (2003). Microtubules provide directional cues for polarized axonal transport through interaction with kinesin motor head. J Cell Biol *162*, 1045-1055.

Nakata, T., and Hirokawa, N. (2007). Neuronal polarity and the kinesin superfamily proteins. Sci STKE *2007*, pe6.

*Nakata, T., Niwa, S., Okada, Y., Perez, F., and Hirokawa, N. (2011). Preferential binding of a kinesin-1 motor to GTP-tubulin-rich microtubules underlies polarized vesicle transport. J Cell Biol *194*, 245-255.

Navone, F., Consalez, G.G., Sardella, M., Caspani, E., Pozzoli, O., Frassoni, C., Morlacchi, E., Sitia, R., Sprocati, T., and Cabibbo, A. (2001). Expression of KIF3C kinesin during neural development and in vitro neuronal differentiation. J Neurochem *77*, 741-753.

Nguyen, H.L., Chari, S., Gruber, D., Lue, C.M., Chapin, S.J., and Bulinski, J.C. (1997). Overexpression of full- or partial-length MAP4 stabilizes microtubules and alters cell growth. J Cell Sci *110 (Pt 2)*, 281-294.

Nishimura, T., Kato, K., Yamaguchi, T., Fukata, Y., Ohno, S., and Kaibuchi, K. (2004). Role of the PAR-3-KIF3 complex in the establishment of neuronal polarity. Nat Cell Biol *6*, 328-334.

Noda, Y., Niwa, S., Homma, N., Fukuda, H., Imajo-Ohmi, S., and Hirokawa, N. (2012). Phosphatidylinositol 4-phosphate 5-kinase alpha (PIPKalpha) regulates neuronal microtubule depolymerase kinesin, KIF2A and suppresses elongation of axon branches. Proc Natl Acad Sci U S A *109*, 1725-1730.

Noda, Y., Okada, Y., Saito, N., Setou, M., Xu, Y., Zhang, Z., and Hirokawa, N. (2001). KIFC3, a microtubule minus end-directed motor for the apical transport of annexin XIIIb-associated Triton-insoluble membranes. J Cell Biol *155*, 77-88.

Nogales, E., Wolf, S.G., and Downing, K.H. (1998). Structure of the alpha beta tubulin dimer by electron crystallography. Nature *391*, 199-203.

Nosten-Bertrand, M., Kappeler, C., Dinocourt, C., Denis, C., Germain, J., Phan Dinh Tuy, F., Verstraeten, S., Alvarez, C., Metin, C., Chelly, J.*, et al.* (2008). Epilepsy in Dcx knockout mice associated with discrete lamination defects and enhanced excitability in the hippocampus. PLoS One *3*, e2473.

Okada, Y., and Hirokawa, N. (1999). A processive single-headed motor: kinesin superfamily protein KIF1A. Science *283*, 1152-1157.

Omori, Y., Chaya, T., Katoh, K., Kajimura, N., Sato, S., Muraoka, K., Ueno, S., Koyasu, T., Kondo, M., and Furukawa, T. (2010). Negative regulation of ciliary length by ciliary male germ cell-associated kinase (Mak) is required for retinal photoreceptor survival. Proc Natl Acad Sci U S A *107*, 22671-22676.

Pagon, R.A., and Daiger, S.P. (1993). Retinitis Pigmentosa Overview. In GeneReviews, R.A. Pagon, T.D. Bird, C.R. Dolan, K. Stephens, and M.P. Adam, eds. (Seattle (WA)).

Palazzo, A., Ackerman, B., and Gundersen, G.G. (2003). Cell biology: Tubulin acetylation and cell motility. Nature *421*, 230.

Paturle-Lafanechere, L., Manier, M., Trigault, N., Pirollet, F., Mazarguil, H., and Job, D. (1994). Accumulation of delta 2-tubulin, a major tubulin variant that cannot be tyrosinated, in neuronal tissues and in stable microtubule assemblies. J Cell Sci *107 (Pt 6)*, 1529-1543.

*Peck, A., Sargin, M.E., LaPointe, N.E., Rose, K., Manjunath, B.S., Feinstein, S.C., and Wilson, L. (2011). Tau isoform-specific modulation of kinesin-driven microtubule gliding rates and trajectories as determined with tau-stabilized microtubules. Cytoskeleton (Hoboken) *68*, 44-55.

Perez, M., Santa-Maria, I., Gomez de Barreda, E., Zhu, X., Cuadros, R., Cabrero, J.R., Sanchez-Madrid, F., Dawson, H.N., Vitek, M.P., Perry, G.*, et al.* (2009). Tau--an inhibitor of deacetylase HDAC6 function. J Neurochem *109*, 1756-1766.

Peterman, E.J., and Scholey, J.M. (2009). Mitotic microtubule crosslinkers: insights from mechanistic studies. Curr Biol *19*, R1089-1094.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem *25*, 1605-1612.

Pfister, K.K., Shah, P.R., Hummerich, H., Russ, A., Cotton, J., Annuar, A.A., King, S.M., and Fisher, E.M. (2006). Genetic analysis of the cytoplasmic dynein subunit families. PLoS Genet *2*, e1.

Polydoro, M., Acker, C.M., Duff, K., Castillo, P.E., and Davies, P. (2009). Age-dependent impairment of cognitive and synaptic function in the htau mouse model of tau pathology. J Neurosci *29*, 10741-10749.

Quelin, C., Saillour, Y., Souville, I., Poirier, K., N'Guyen-Morel, M.A., Vercueil, L., Millisher-Bellaiche, A.E., Boddaert, N., Dubois, F., Chelly, J.*, et al.* (2012). Mosaic DCX deletion causes subcortical band heterotopia in males. Neurogenetics *13*, 367-373.

Reed, N.A., Cai, D., Blasius, T.L., Jih, G.T., Meyhofer, E., Gaertig, J., and Verhey, K.J. (2006). Microtubule acetylation promotes kinesin-1 binding and transport. Curr Biol *16*, 2166-2172.

Reifert, J., Hartung-Cranston, D., and Feinstein, S.C. (2011). Amyloid beta-mediated cell death of cultured hippocampal neurons reveals extensive Tau fragmentation without increased full-length tau phosphorylation. J Biol Chem *286*, 20797-20811.

Reiner, O., Coquelle, F.M., Peter, B., Levy, T., Kaplan, A., Sapir, T., Orr, I., Barkai, N., Eichele, G., and Bergmann, S. (2006). The evolving doublecortin (DCX) superfamily. BMC Genomics *7*, 188.

Saito, N., Okada, Y., Noda, Y., Kinoshita, Y., Kondo, S., and Hirokawa, N. (1997). KIFC2 is a novel neuron-specific C-terminal type kinesin superfamily motor for dendritic transport of multivesicular body-like organelles. Neuron *18*, 425-438.

Santarella, R.A., Skiniotis, G., Goldie, K.N., Tittmann, P., Gross, H., Mandelkow, E.M., Mandelkow, E., and Hoenger, A. (2004). Surface-decoration of microtubules by human tau. J Mol Biol *339*, 539-553.

Sapir, T., Horesh, D., Caspi, M., Atlas, R., Burgess, H.A., Wolf, S.G., Francis, F., Chelly, J., Elbaum, M., Pietrokovski, S.*, et al.* (2000). Doublecortin mutations cluster in evolutionarily conserved functional domains. Hum Mol Genet *9*, 703-712.

Saragoni, L., Hernandez, P., and Maccioni, R.B. (2000). Differential association of tau with subsets of microtubules containing posttranslationally-modified tubulin variants in neuroblastoma cells. Neurochem Res *25*, 59-70.

Schaar, B.T., Kinoshita, K., and McConnell, S.K. (2004). Doublecortin microtubule affinity is regulated by a balance of kinase and phosphatase activity at the leading edge of migrating neurons. Neuron *41*, 203-213.

Seidel, C., Zekert, N., and Fischer, R. (2012). The Aspergillus nidulans kinesin-3 tail is necessary and sufficient to recognize modified microtubules. PLoS One *7*, e30976.

Seitz, A., Kojima, H., Oiwa, K., Mandelkow, E.M., Song, Y.H., and Mandelkow, E. (2002). Single-molecule investigation of the interference between kinesin, tau and MAP2c. EMBO J *21*, 4896-4905.

Setou, M., Nakagawa, T., Seog, D.H., and Hirokawa, N. (2000). Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. Science *288*, 1796-1802.

*Setou, M., Seog, D.H., Tanaka, Y., Kanai, Y., Takei, Y., Kawagishi, M., and Hirokawa, N. (2002). Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. Nature *417*, 83-87.

Sharp, D.J., Rogers, G.C., and Scholey, J.M. (2000). Roles of motor proteins in building microtubule-based structures: a basic principle of cellular design. Biochim Biophys Acta *1496*, 128-141.

Shi, S.H., Cheng, T., Jan, L.Y., and Jan, Y.N. (2004). APC and GSK-3beta are involved in mPar3 targeting to the nascent axon and establishment of neuronal polarity. Curr Biol *14*, 2025-2032.

Shirasu, M., Kimura, K., Kataoka, M., Takahashi, M., Okajima, S., Kawaguchi, S., Hirasawa, Y., Ide, C., and Mizoguchi, A. (2000). VAMP-2 promotes neurite elongation and SNAP-25A increases neurite sprouting in PC12 cells. Neurosci Res *37*, 265-275.

*Silverman, M.A., Kaech, S., Ramser, E.M., Lu, X., Lasarev, M.R., Nagalla, S., and Banker, G. (2010). Expression of kinesin superfamily genes in cultured hippocampal neurons. Cytoskeleton (Hoboken) *67*, 784-795.

Small, S.A., and Duff, K. (2008). Linking Abeta and tau in late-onset Alzheimer's disease: a dual pathway hypothesis. Neuron *60*, 534-542.

*Song, A.H., Wang, D., Chen, G., Li, Y., Luo, J., Duan, S., and Poo, M.M. (2009). A selective filter for cytoplasmic transport at the axon initial segment. Cell *136*, 1148-1160.

Soppina, V., Herbstman, J.F., Skiniotis, G., and Verhey, K.J. (2012). Luminal localization of alpha-tubulin K40 acetylation by cryo-EM analysis of fab-labeled microtubules. PLoS One *7*, e48204.

Sossey-Alaoui, K., Hartung, A.J., Guerrini, R., Manchester, D.K., Posar, A., Puche-Mira, A., Andermann, E., Dobyns, W.B., and Srivastava, A.K. (1998). Human doublecortin (DCX) and the homologous gene in mouse encode a putative Ca2+-dependent signaling protein which is mutated in human X-linked neuronal migration defects. Hum Mol Genet *7*, 1327-1332.

Spiliotis, E.T., Hunt, S.J., Hu, Q., Kinoshita, M., and Nelson, W.J. (2008). Epithelial polarity requires septin coupling of vesicle transport to polyglutamylated microtubules. J Cell Biol *180*, 295-303.

*Stamer, K., Vogel, R., Thies, E., Mandelkow, E., and Mandelkow, E.M. (2002). Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. J Cell Biol *156*, 1051-1063.

Stokin, G.B., Lillo, C., Falzone, T.L., Brusch, R.G., Rockenstein, E., Mount, S.L., Raman, R., Davies, P., Masliah, E., Williams, D.S*., et al.* (2005). Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. Science *307*, 1282-1288.

Stone, M.C., Roegiers, F., and Rolls, M.M. (2008). Microtubules have opposite orientation in axons and dendrites of Drosophila neurons. Mol Biol Cell *19*, 4122-4129.

Stoothoff, W., Jones, P.B., Spires-Jones, T.L., Joyner, D., Chhabra, E., Bercury, K., Fan, Z., Xie, H., Bacskai, B., Edd, J*., et al.* (2009). Differential effect of three-repeat and four-repeat tau on mitochondrial axonal transport. J Neurochem *111*, 417-427.

*Sung, H.H., Telley, I.A., Papadaki, P., Ephrussi, A., Surrey, T., and Rorth, P. (2008). Drosophila ensconsin promotes productive recruitment of Kinesin-1 to microtubules. Dev Cell *15*, 866-876.

Takei, Y., Teng, J., Harada, A., and Hirokawa, N. (2000). Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes. J Cell Biol *150*, 989-1000.

Takemura, R., Okabe, S., Umeyama, T., Kanai, Y., Cowan, N.J., and Hirokawa, N. (1992). Increased microtubule stability and alpha tubulin acetylation in cells transfected with microtubule-associated proteins MAP1B, MAP2 or tau. J Cell Sci *103 (Pt 4)*, 953-964.

Tapia, M., Wandosell, F., and Garrido, J.J. (2010). Impaired function of HDAC6 slows down axonal growth and interferes with axon initial segment development. PLoS One *5*, e12908.

Taylor, K.R., Holzer, A.K., Bazan, J.F., Walsh, C.A., and Gleeson, J.G. (2000). Patient mutations in doublecortin define a repeated tubulin-binding domain. J Biol Chem *275*, 34442-34450.

Teng, J., Takei, Y., Harada, A., Nakata, T., Chen, J., and Hirokawa, N. (2001). Synergistic effects of MAP2 and MAP1B knockout in neuronal migration, dendritic outgrowth, and microtubule organization. J Cell Biol *155*, 65-76.

Terwel, D., Lasrado, R., Snauwaert, J., Vandeweert, E., Van Haesendonck, C., Borghgraef, P., and Van Leuven, F. (2005). Changed conformation of mutant Tau-P301L underlies the moribund tauopathy, absent in progressive, nonlethal axonopathy of Tau-4R/2N transgenic mice. J Biol Chem *280*, 3963-3973.

Thinakaran, G., and Koo, E.H. (2008). Amyloid precursor protein trafficking, processing, and function. J Biol Chem *283*, 29615-29619.

Thorn, K.S., Ubersax, J.A., and Vale, R.D. (2000). Engineering the processive run length of the kinesin motor. J Cell Biol *151*, 1093-1100.

Tien, N.W., Wu, G.H., Hsu, C.C., Chang, C.Y., and Wagner, O.I. (2011). Tau/PTL-1 associates with kinesin-3 KIF1A/UNC-104 and affects the motor's motility characteristics in C. elegans neurons. Neurobiol Dis *43*, 495-506.

Tilney, L.G., Bryan, J., Bush, D.J., Fujiwara, K., Mooseker, M.S., Murphy, D.B., and Snyder, D.H. (1973). Microtubules: evidence for 13 protofilaments. J Cell Biol *59*, 267-275.

*Tint, I., Jean, D., Baas, P.W., and Black, M.M. (2009). Doublecortin associates with microtubules preferentially in regions of the axon displaying actin-rich protrusive structures. J Neurosci *29*, 10995-11010.

Tischfield, M.A., and Engle, E.C. (2010). Distinct alpha- and beta-tubulin isotypes are required for the positioning, differentiation and survival of neurons: new support for the 'multi-tubulin' hypothesis. Biosci Rep *30*, 319-330.

Trinczek, B., Ebneth, A., Mandelkow, E.M., and Mandelkow, E. (1999). Tau regulates the attachment/detachment but not the speed of motors in microtubule-dependent transport of single vesicles and organelles. J Cell Sci *112 (Pt 14)*, 2355-2367.

Tsai, J.W., Lian, W.N., Kemal, S., Kriegstein, A.R., and Vallee, R.B. (2010). Kinesin 3 and cytoplasmic dynein mediate interkinetic nuclear migration in neural stem cells. Nat Neurosci *13*, 1463-1471.

Uchida, T., Akiyama, H., Sakamoto, W., Koga, T., Yan, K., Uchida, C., Hirose, K., and Itoh, T.J. (2009). Direct optical microscopic observation of the microtubule polymerization intermediate sheet structure in the presence of gas7. J Mol Biol *391*, 849-857.

Utton, M.A., Noble, W.J., Hill, J.E., Anderton, B.H., and Hanger, D.P. (2005). Molecular motors implicated in the axonal transport of tau and alpha-synuclein. J Cell Sci *118*, 4645-4654.

Vale, R.D., Coppin, C.M., Malik, F., Kull, F.J., and Milligan, R.A. (1994). Tubulin GTP hydrolysis influences the structure, mechanical properties, and kinesin-driven transport of microtubules. J Biol Chem *269*, 23769-23775.

Verhey, K.J., and Gaertig, J. (2007). The tubulin code. Cell Cycle *6*, 2152-2160.

Verhey, K.J., and Hammond, J.W. (2009). Traffic control: regulation of kinesin motors. Nat Rev Mol Cell Biol *10*, 765-777.

*Vershinin, M., Carter, B.C., Razafsky, D.S., King, S.J., and Gross, S.P. (2007). Multiple-motor based transport and its regulation by Tau. Proc Natl Acad Sci U S A *104*, 87-92.

*von Massow, A., Mandelkow, E.M., and Mandelkow, E. (1989). Interaction between kinesin, microtubules, and microtubule-associated protein 2. Cell Motil Cytoskeleton *14*, 562-571.

Vreugdenhil, E., Kolk, S.M., Boekhoorn, K., Fitzsimons, C.P., Schaaf, M., Schouten, T., Sarabdjitsingh, A., Sibug, R., and Lucassen, P.J. (2007). Doublecortin-like, a microtubule-associated protein expressed in radial glia, is crucial for neuronal precursor division and radial process stability. Eur J Neurosci *25*, 635-648.

Wade, R.H. (2009). On and around microtubules: an overview. Mol Biotechnol *43*, 177-191.

Wagner, W., Brenowitz, S.D., and Hammer, J.A., 3rd (2011). Myosin-Va transports the endoplasmic reticulum into the dendritic spines of Purkinje neurons. Nat Cell Biol *13*, 40-48.

*Walter, W.J., Beranek, V., Fischermeier, E., and Diez, S. (2012). Tubulin acetylation alone does not affect kinesin-1 velocity and run length in vitro. PLoS One *7*, e42218.

Wordeman, L. (2005). Microtubule-depolymerizing kinesins. Curr Opin Cell Biol *17*, 82-88.

Xu, Y., Takeda, S., Nakata, T., Noda, Y., Tanaka, Y., and Hirokawa, N. (2002). Role of KIFC3 motor protein in Golgi positioning and integration. J Cell Biol *158*, 293-303.

*Yajima, H., Ogura, T., Nitta, R., Okada, Y., Sato, C., and Hirokawa, N. (2012). Conformational changes in tubulin in GMPCPP and GDP-taxol microtubules observed by cryoelectron microscopy. J Cell Biol *198*, 315-322.

Yamazaki, H., Nakata, T., Okada, Y., and Hirokawa, N. (1995). KIF3A/B: a heterodimeric kinesin superfamily protein that works as a microtubule plus end-directed motor for membrane organelle transport. J Cell Biol *130*, 1387-1399.

Yang, Z., and Goldstein, L.S. (1998). Characterization of the KIF3C neural kinesin-like motor from mouse. Mol Biol Cell *9*, 249-261.

Yang, Z., Roberts, E.A., and Goldstein, L.S. (2001). Functional analysis of mouse C-terminal kinesin motor KifC2. Mol Cell Biol *21*, 2463-2466.

Ye, F., Tan, L., Yang, Q., Xia, Y., Deng, L.W., Murata-Hori, M., and Liou, Y.C. (2011). HURP regulates chromosome congression by modulating kinesin Kif18A function. Curr Biol *21*, 1584-1591.

Yonekawa, Y., Harada, A., Okada, Y., Funakoshi, T., Kanai, Y., Takei, Y., Terada, S., Noda, T., and Hirokawa, N. (1998). Defect in synaptic vesicle precursor transport and neuronal cell death in KIF1A motor protein-deficient mice. J Cell Biol *141*, 431-441.

Yoshimura, Y., Terabayashi, T., and Miki, H. (2010). Par1b/MARK2 phosphorylates kinesin-like motor protein GAKIN/KIF13B to regulate axon formation. Mol Cell Biol *30*, 2206-2219.

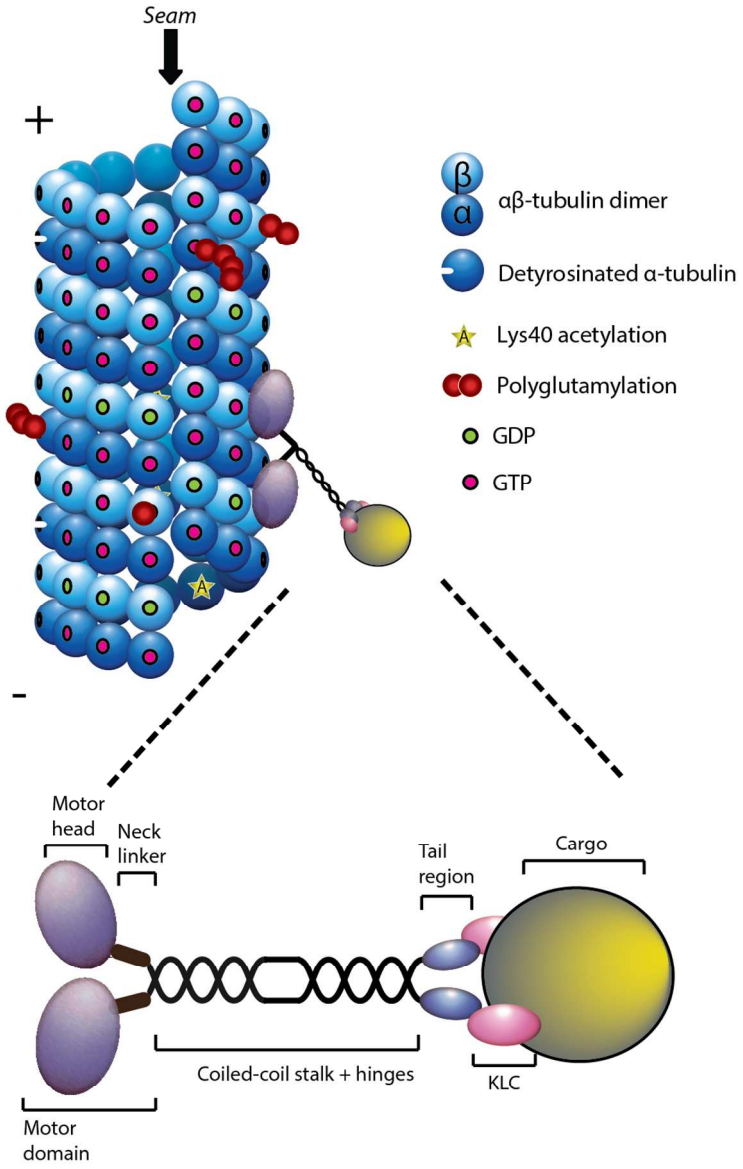
Yu, Y., and Feng, Y.M. (2010). The role of kinesin family proteins in tumorigenesis and progression: potential biomarkers and molecular targets for cancer therapy. Cancer *116*, 5150-5160.

Yuan, A., Kumar, A., Peterhoff, C., Duff, K., and Nixon, R.A. (2008). Axonal transport rates in vivo are unaffected by tau deletion or overexpression in mice. J Neurosci *28*, 1682-1687.

Zempel, H., Thies, E., Mandelkow, E., and Mandelkow, E.M. (2010). Abeta oligomers cause localized Ca(2+) elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. J Neurosci *30*, 11938-11950.

Zhang, Y., Kwon, S., Yamaguchi, T., Cubizolles, F., Rousseaux, S., Kneissel, M., Cao, C., Li, N., Cheng, H.L., Chua, K.*, et al.* (2008). Mice lacking histone deacetylase 6 have hyperacetylated tubulin but are viable and develop normally. Mol Cell Biol *28*, 1688-1701.

Human Kif	Sub-family	Uniprot	Tail-less motor axon/dendrite preference ≥stage 3 neurons	Full-length preference ≥stage 3 neurons	% motor sequence identity
Kif5A	1	Q12840	Axonal[7, 18]	Non-selective [e.g. 18,10]	100
Kif5B	1	P33176	Axonal[7, 18]	Non-selective [e.g. 18]	85
Kif5C	1	O60282	Axonal[7, 19, 8, 4, 18]	Non-selective [e.g. 18, 10]	91
Kif3A	2	Q9Y496	Axonal as a dimer with Kif3B, weakly axonal as a dimer with Kif3C[7]	Non-selective[17]Weakly axonal[12]	47
Kif3B	2	O15066	See above[7]	Non-selective[17, 25]	44
Kif3C	2	O14782	See above[7]	Cell type dependent[26,] Non-selective[17, 20]	42
Kif17	2	Q9P2E2	Axonal[7] Non-selective [18, 24]	Dendritic[2, 23, 3, 11, 24]	46
Kif1A	3	Q12756	Non-selective[7, 19, 8, 13]	Non-selective[9]	43
Kif1Bα	3	O60333-3	Non-selective [7]	Non-selective[16]	44
Kif1Bβ	3	O60333-2	Non-selective[7]	Non-selective[1]	44
Kif13A	3	Q9H1H9	Axonal[7]	Non-selective[9]	44
Kif21A	4	Q7Z4S6	Axonal[7]	Non-selective[15]Axonal[14]	39
Kif21B	4	O75037	Non-selective[7]	Dendrites[15]	42
Kif2A	13	O00139	?	Non-selective[21]	38
KifC2	14	Q96AC6	?	Dendritic[22], Non-selective[5]	32
KifC3	14	Q9BVG8	?	Axonal/*synaptic*[6]	37



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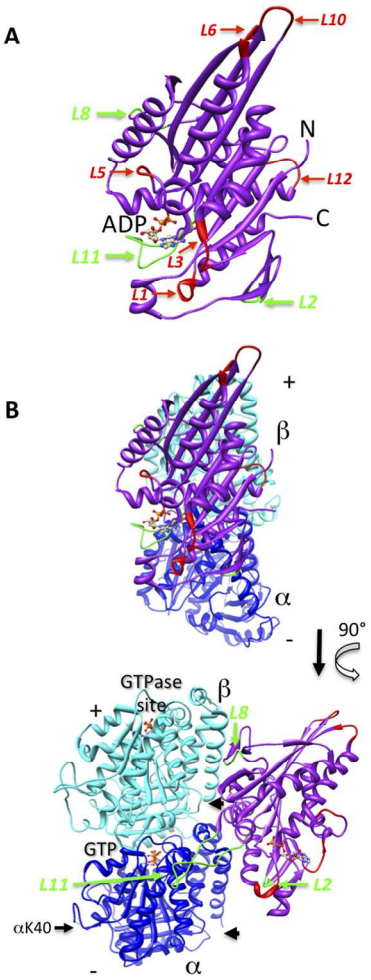
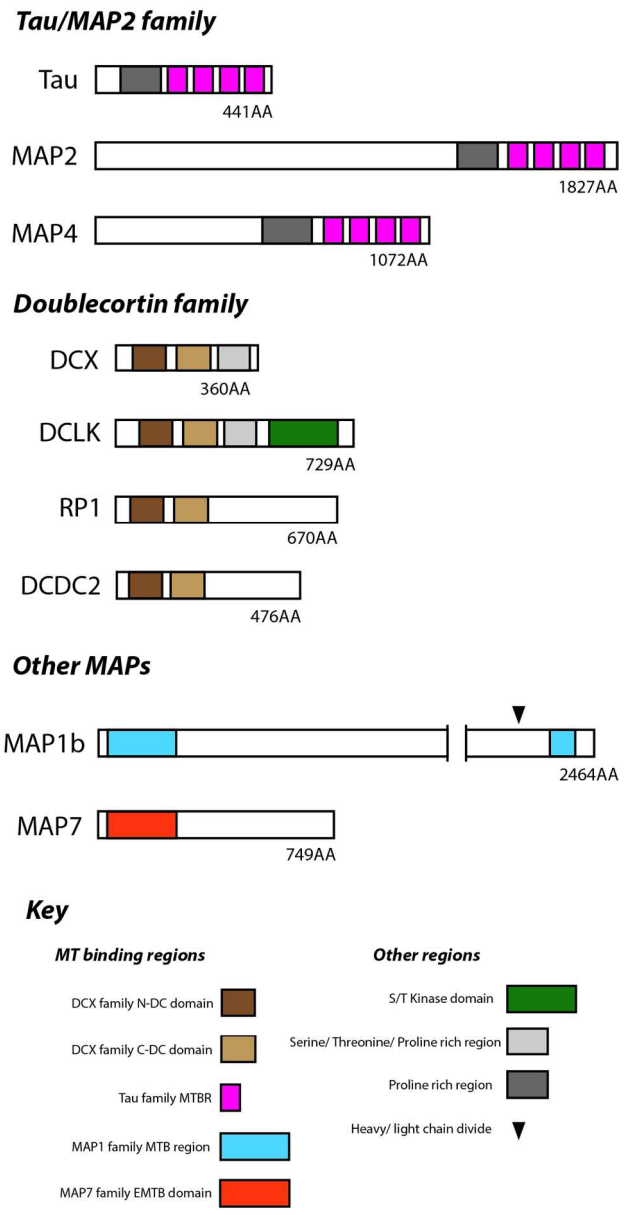
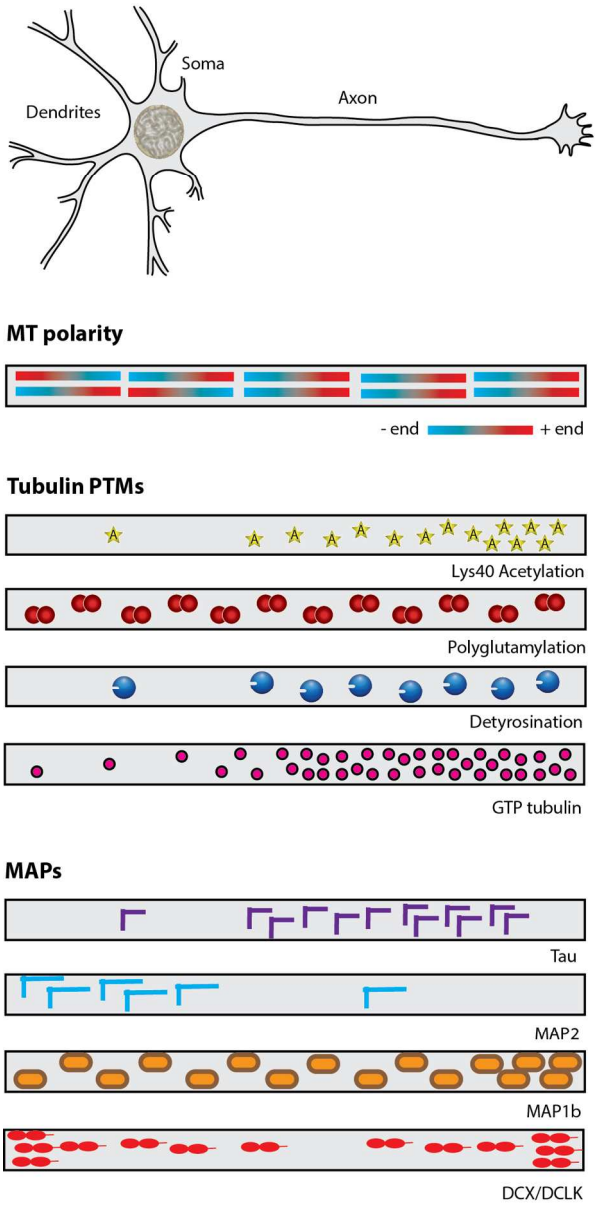


209x297mm (150 x 150 DPI)



95x186mm (300 x 300 DPI)



91x185mm (300 x 300 DPI)